DIRECT AND INDIRECT ECOLOGICAL INTERACTIONS BETWEEN AQUACULTURE ACTIVITIES AND MARINE FISH COMMUNITIES IN SCOTLAND

BY

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DECLARATION

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree.

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ABSTRACT

Presence of coastal aquaculture activities in marine landscapes is growing. However, there is insufficient knowledge on the subsequent ecological interactions between these activities and marine fish communities. The overall aim of this thesis was to evaluate the direct and indirect ecological effects of aquaculture activities on marine fish communities in Scotland. A combination of empirical and modelling approaches was employed to collect evidence of how aquaculture activities affect marine fish communities at the individual, population and ecosystem levels around coastal sea cages.

The two fish farms evaluated in this research provided the wild fish sampled near the sea cages with a habitat rich in food resources which is reflected in an overall better biological condition. Results of the stomach content analysis indicated that mackerel (*Scomber scombrus*), whiting (*Merlangius merlangus*) and saithe (*Pollachius virens*) sampled near sea cages consumed wasted feed which was also reflected in their modified FA profiles. The overall effects of the two fish farms were more pronounced in young whiting and saithe than in mixed aged mackerel sampled near the sea cages.

The phase space modelling approach indicated that the overall potential for fish farms to act at the extremes as either population sources (a habitat that is rich in resources and leads to an overall improved fitness) or ecological traps (a habitat that appears to be rich in resources but is not and leads to an overall poor fitness) are higher for juvenile whiting than for mackerel. Based on the empirical evidence and literature the two fish farms are more likely to be a population source for wild fishes.

Using an ecosystem modelling approach indicated that fish farming impacts the food web in a sea loch via nutrient loading. Mussel farming relies on the natural food resources and has the potential to affect the food web in a sea loch via competing with zooplankton for resources which can affect higher trophic levels. The presence of both activities can balance the overall impact in a sea loch as compared to the impact induced if each of these activities were present on their own. Both activities have the potential do induce direct and indirect effects on the wild fish and the entire sea loch system.

The results of this PhD identified several gaps in data and thus could be used to improve future sampling designs. It is important to evaluate the cumulative effect of the presence of aquaculture activities in terms of nutrient loading and physical structure in the environment. Using a combination of empirical and modelling approaches is

recommended to gain further insight into the ecological impacts of aquaculture activities on wild fish communities.

Results of this PhD study could lead to more informed decisions in managing the coastal aquaculture activities. Establishing coastal fish farms as aquatic sanctuaries can be of an advantage to increase fish production and conserve species that are endangered provided that no commercial and recreational fishing is allowed nearby. It would be useful to have long term monitoring of the fish stocks around the cages and if there is any production at the regional level. Additionally, information on behaviour, migration patterns should be collected to understand the impacts of aquaculture activities on fish stocks. From an aquaculture perspective, ecologically engineered fish farms in addition to careful site selection in new aquaculture developments may improve nutrient loading into the ecosystem.

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CHAPTER 1
COASTAL AQUACULTURE ACTIVITIES AND MARINE FISH COMMUNITIES

1.1 Introduction

Artificial structures for commercial or recreational purposes are increasingly common in coastal areas (see Dugan et al. 2011; Alexander et al. 2012; Dafforn et al. 2015). There are growing demands for artificial structures such as marine energy installations (traditional gas or oil; or renewables), artificial reefs, fish aggregating devices and coastal aquaculture structures (e.g. sea cages, mussel rafts, algae longlines). Building of artificial structures for coastal protection (e.g. breakwaters, seawalls, jetties etc.) to support growing populations in coastal areas and the potential threats from climate changes (e.g. sea-level rise, extreme weather) are also on the rise (Dugan et al. 2011; Dafforn et al. 2015). Although presence of artificial structures in aquatic environments is common the ecological consequences are not sufficiently understood (Bulleri and Chapman 2010; Dugan et al. 2011).

In this thesis, I explored the direct and indirect ecological interactions between two coastal aquaculture activities and marine fish communities on the West Coast of Scotland. Amongst a number of potential direct ecological impacts (e.g. disruption of migratory routes, exposure to diseases and pollutants), I evaluated the direct impacts of two fish farms on the physiology of three wild fish species sampled around the sea cages. A combination of positive (e.g. improved reproductive output) and negative (e.g. increase in predation) effects of fish farming were evaluated at the population level of wild fish visiting the sea cages. As species do not live in isolation they interact with other species creating complex networks and thus any anthropogenic change such as fish farming that affects a fish population can have potential knock-on effects on other species (e.g. Estes et al. 2011). Thus, an ecosystem-based approach was also undertaken to evaluate direct and indirect coastal aquaculture impacts on the ecosystem with focus on wild fish visiting the sea cages.

I used a combination of empirical (fieldwork and laboratory analysis) and modelling (statistical and mathematical) approaches to gain a more holistic understanding

of aquaculture effects on pelagic and benthopelagic fish communities at the vicinities of sea cages. Empirical work was conducted to collect data that would allow the detection of impacts of two fish farms on the diet and biological condition of wild non-salmonid fish caught near and away from sea cages. The methodologies used to collect data can be found in Chapters 2-3 and the results of these are found in Chapters 4-6. The empirical data collected, and additional data collected from the literature were used to build models to understand and extrapolate impacts of fish farming at the population (see Chapter 7) and ecosystem levels (see Chapter 8). Based on these results, I weighed up possible positive and negative effects of two fish farms on wild fish communities in Chapter 9. Concluding remarks and future directions are also included in Chapter 9.

In this chapter, I provide essential context needed for the research undertaken in this thesis and its significance. To understand the impacts of sea cages on wild fish communities we need to find evidence for attraction (section 1.2) and reasons for this attraction (section 1.3). The attracted fish can be positively or negatively affected or unaffected by fish farming (section 1.4) and weighing up of these effects is presented in section 1.5. Fish farming can affect the physiology of commercially important wild fish species with subsequent impacts on commercial and recreational fishing (section 1.6). Wild fish around fish farms can also pose benefits and costs to the fish farming industry (section 1.7). There is lack of knowledge on ecological interactions between the fish farming industry and wild marine fish communities in Scotland. An overview of the current state of capture fisheries and aquaculture in Scotland is presented in section 1.8. Fish species of interest and objectives of this PhD thesis are described in sections 1.9 and 1.10, respectively.

1.2 Coastal aquaculture activities and wild fish communities

Artificial (e.g. oil platforms, fish aggregating devices, artificial reefs) and natural (e.g. jellyfish, drifting algae, free-floating logs) objects in marine environments can attract fish (Pickering and Whitmarsh 1997; Claisse et al. 2014; Reubens et al. 2014). These structures can create floating and fixed habitats for various organisms. Coastal fish farming takes place in sea cages that can also attract fish (e.g. Dempster et al. 2002). However, one of the main differences between other artificial structures (e.g. oil platforms, artificial reefs) and coastal sea cages is the considerable amount of effluent generated by the fish farming activities.

Production of fish in sea cages results in large amounts of organic by-products in the form of particulate matter originating from uneaten food and faeces, dissolved metabolic waste including ammonia and urea excreted from the gills and organic matter resulting from scraping of biofouling on cages (reviewed by Holmer 2010; Uglem et al. 2014; Price et al. 2015). Nutrient emission from fish farms can have a range of ecological impacts on the surrounding aquatic environment such as local eutrophication, impacts on benthic fauna and local wild fish populations (see Mente et al. 2006; Holmer 2010; Uglem et al. 2014).

1.2.1 Evidence for wild fish attraction to aquaculture structures

A number of studies reported that aquaculture in net cages located in coastal areas, lakes, or reservoirs affect the presence and abundance of wild fish in their vicinities (reviewed by Sanchez-Jerez et al. 2011; Demétrio et al. 2012). The majority of these studies were conducted in coastal marine waters and predominantly around fish farms in the Mediterranean Sea and Norwegian coast (Sanchez-Jerez et al. 2011). Wild fish attraction to sea cages has been reported in Spain (Dempster et al. 2002, 2004; Boyra et al. 2004; Tuya et al. 2006), Greece (Machias et al. 2006), Turkey (Akyol and Ertosluk 2010), the Adriatic Sea (Šegvić Bubić et al. 2011), Red Sea (Özgül and Angel 2013), United States (US) (Oakes and Pondella 2009), Indonesia (Sudirman et al. 2009), Australia (Dempster et al. 2004; Felsing et al. 2005), Norway (Bjordal and Skar 1992; Dempster et al. 2009, 2010, 2011), and Scotland (Carss 1990).

Many fish species have been noted near coastal marine fish farms (Sanchez-Jerez et al. 2011). Wild fish have been attracted to fish farms of more than 10 cultured fish species including Atlantic salmon (*Salmo salar*), sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) amongst others (reviewed by Uglem et al. 2014). Wild fish are also attracted to other aquaculture sturctures such as shellfish rafts and longline mussel farms (Laffargue et al. 2006; Morrisey et al. 2006).

Although the majority of studies conducted in various parts of the world have detected aquaculture effects on wild fish populations some studies have reported no apparent aquaculture impacts (e.g. Mente et al. 2008; Tanner and Williams 2015). For example, Tanner and Williams (2015) using baited remote underwater camera reported no apparent impacts of yellowtail kingfish (*Seriola lalandi*) farming on resident benthic fish and crustaceans in Fitzgerald Bay, Australia. The researchers suggested that the lack

of aquaculture effects on benthic organisms in the bay might be related to the low aquaculture activity in the area and the high rates of water movement which results in fish farm waste dilution and dispersion. Similarly, Mente et al. (2008) evaluated the diet composition of several commercially important fish (e.g. haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), and flatfishes) near and away from fish farms located in Scottish lochs. Any dietary differences between lochs or sites near and away from farms were not related to fish farming. It is worth noting that the average fish mass near fish farms was greater than the corresponding fish from reference sites. The authors suggested the need for further investigations as the sampling was conducted at distances greater than 50 m from the nearest fish farm (Mente et al. 2008).

1.3 Why are fish attracted to coastal sea cages?

Coastal fish farms attract fish for a combination of reasons such as trophic resources (e.g. uneaten food pellets (Dempster et al. 2002, 2011; Fernandez-Jover et al. 2007a, 2011a; Uglem et al. 2014) or increase in food (see Sanchez-Jerez et al. 2011)), shelter (see Uglem et al. 2014), chemical cues (e.g. amino acids) produced by farmed fish (Dempster et al. 2002), artificial light (McConnell et al. 2010; Otterå and Skilbrei 2014), noise (Arechavala-Lopez et al. 2010 and references therein). The fish composition around fish farms depends on other factors such as depth, coastal topographic complexity, distance from coast, currents, composition of fish in nearby waters, and the farmed fish and composition of feed (Dempster et al. 2002; Fernandez-Jover et al. 2008).

1.3.1 Food availability: natural and artificial

Sanchez-Jerez et al. (2011) suggested that fish farms might provide a higher quality habitat in terms of food and protection than habitats of good quality such as artificial reefs. Culture of fish in sea cages can enrich the waters with organic (e.g. faeces, waste feed) and inorganic material (e.g. ammonia) (e.g. Holmer 2010; Price et al. 2015). There is insufficient knowledge on exact amounts of nutrient enrichment from fish farms in the forms of waste feed and faecal material from undigested food because it is challenging to distinguish waste feed from other solid wastes (Islam 2005). It is also worth noting that information on the amount of lost feed is difficult to obtain because of commercial sensitivity. It is estimated that the amount of uneaten feed can range from 1 to 20% depending on farm, cultured species, stocking density, feeding regimen and other factors

such as weather conditions (Islam 2005; Dempster and Sanchez-Jerez 2008). Uglem et al. (2014), assuming up to 5% waste feed (Otterå et al. 2009), estimated that tens of thousands of tonnes of feed is available for wild fish visiting fish farms in Norway.

Food availability around the sea cages attracts a number of fish species and some of these fishes consume waste feed lost through the cages. In their review, Uglem et al. (2014) noted that more than 17 wild fish species have been reported to consume waste feed from fish farms located in Scotland, Norway, Spain, Indonesia and Brazil. Izquierdo-Gómez et al. (2015) reported that fish from different trophic levels (e.g. zooplanktivorous, piscivorous) feed directly on waste feed from fish farm or indirectly through predation on aggregated prey.

Increased supply of dissolved nutrients from fish farms to the aquatic environment can potentially lead to increase in phytoplankton growth (Islam 2005; Price et al. 2015) which can be a source of food for zooplankton. Young fish are attracted to high levels of zooplankton which in turn attracts bigger predatory fish (Sanchez-Jerez et al. 2011). However, it is worth noting that several studies report no relationship between increase in nutrient effluent from fish farms and phytoplankton growth (Price et al. 2015).

Production of fish takes place in a complex assemblage of structures such as nets, cages, floats and ropes which lead to settlement and growth of marine algae and animals (also known as biofouling) which in turn can be consumed by other aquatic organisms (Sanchez-Jerez et al. 2011; Fitridge et al. 2012). The increase in nutrient effluent from fish farms can potentially increase growth of biofouling communities (Sanchez-Jerez et al. 2011).

Both waste feed and structural complexity of sea cages attract different fish with waste feed being the stronger factor of the two factors (Tuya et al. 2006). For example, Tuya et al. (2006) reported that when fish farming activity stopped the abundance of wild fish lowered from about 50 fold higher than areas with no fish farming to less than 2 fold when only the fish farming infrastructure remained. Similar observations have been noted around fish farms in Scotland by Dr. Tom Wilding (The Scottish Association for Marine Science (SAMS), pers. comm., January 2017). Tuya et al. (2006) suggested that the infrastructure of fish farms play a weaker role in attracting wild fish than the waste feed and presence of cultured fish. It is worth noting that the infrastructural complexity of the fish farms provides shelter and food (e.g. algae and sessile invertebrates) for some fish species (Tuya et al. 2006).

Daily feeding activities of cultured fish can affect the behaviour of wild fish around fish farms with greatest numbers of wild fish occurring during feeding times (Sudirman et al. 2009; Bacher et al. 2015). Similar results were reported by Ballester-Moltó et al. (2015) who also added that wild fish around fish farms show similar behaviour in anticipating food as that found in farmed fish. Uglem et al. (2009) studied the movement patterns of tagged saithe around salmon farms in Norway and broadly related the movement patterns around farms with feeding times.

Wild fish are often found in greatest numbers immediately beneath the cages where the waste feed is highest (Dempster et al. 2010). Dempster et al. (2010) also noted that the level of aggregation around fish farms is related to the different behaviour of species and location of the fish farms. For example, saithe (*Pollachius virens*) is a pelagic feeder often found in close association to fish farms and also found to consume high quantities of waste feed (over 75% of the diet) whereas cod (*Gadus morhua*) which is a benthic feeder is found more dispersed around fish farms which corresponded to lower waste feed consumption (about 30% of the diet) (Dempster et al. 2010).

Sea cage farming affects the spatiotemporal distribution of wild fish in warm oligotrophic environments (e.g. Meditteranean Sea) (Giannoulaki et al. 2005; Machias et al. 2005) and nutrient-rich environments (e.g. coasts of Canada, United Kingdom) (Goodbrand et al. 2013). In the Mediterranean Sea, the increase in wild fish biomass beyond the sea cages was attributed to low nutrient levels and low primary productivity and in limited secondary production (Machias et al. 2005). On the other hand, Goodbrand et al. (2013), using hydroacoustic survey methods, reported that in nutrient rich environments such as the Canadian coast, fish farming can potentially have significant ecosystem level impacts. This occurs via the consumption of waste feed by resident wild fish which in turn attract predators that would then move between different farm locations because of rise in competition or predation (Goodbrand et al. 2013). Goodbrand et al. (2013) and Dempster et al. (2009) did not correlate the increase in biological activity around fish farms with the amount of waste feed. Both studies used proxies (number of sea cages (Goodbrand et al. 2013) and stocking densities (Dempster et al. 2009)) to estimate the amount of waste feed from fish farms. Dempster et al. (2009) noted that there was variation in wild fish biomass near fish farms either because the amount of food input into fish farms is not related to the waste feed or that some farms are located near locations rich in wild fish. Goodbrand et al. (2013) suggested that bottom-up effects counteract the impacts of the amount of waste feed from fish farms.

1.3.2 Other factors attracting fish

The reasons why fish are attracted to floating objects remain poorly understood. Several mechanisms have been proposed to explain the attraction of fish to floating objects (Fréon and Dagorn 2000; Castro et al. 2002). In their review of associative behaviour of fish to floating objects, Fréon and Dagorn (2000) noted that the meeting point hypothesis offers the most suitable explanation for this behaviour. The meeting point hypothesis suggests that floating objects act as meeting points for individuals or small schools. This leads to the formation of bigger schools that would increase the survival rate of individuals by using group as a refuge and better ability to find food (Dagorn and Fréon 1999). Additionally, Fréon and Dagorn (2000) noted in their review that the indicator-log hypothesis (Hall 1992) also gives a suitable explanation for the behaviour. Based on the indicator-log hypothesis fish (e.g. tuna) use natural floating objects as an indicator for habitats rich in resources because many of these objects originate from areas of high productivity (e.g. river mouths, mangrove swamps or in frontal zones and convergences) where high amounts of planktonic food accumulate (Fréon and Dagorn 2000). Although, these hypotheses explain the behaviour of larger species such as tuna other mechanisms have been suggested to explain the attraction of larval and juvenile fishes to floating objects such as protection from predators, increased food availability, and transport to suitable habitats for settlement (see Castro et al. 2002; Dempster and Taquet 2004).

Fish may also be attracted to fish farms because of the noise and artificial lighting in sea cages (see Arechavala-Lopez et al. 2010 and references therein; McConnell et al. 2010; Otterå and Skilbrei 2014). McConnell et al. (2010) using an experimental setup studied the effects of lights on fish abundance in coastal British Columbia and found a significant increase in larvae, juvenile and adult fish near underwater light as compared to control site. Artificial light, used in salmon farming to delay fish maturation, also attracts zooplankton which increases the food availability around the sea cages (McConnell et al. 2010).

Similar to fish aggregating devices, sea cages can attract fish because of visual and olfactory (e.g. fouling organisms on the structures and other wild organism can produce chemicals) cues as well as sound and vibrations from other wild fish around the cages or the cages themselves (Dempster and Kingsford 2003; Dempster and Taquet 2004).

A number of studies report temporal and spatial variations in composition and structure of wild fish assemblages around coastal fish farms (e.g. Dempster et al. 2002, 2009; Valle et al. 2007). Seasonal and reproductive migrations patterns also affect the abundance of fish around sea cages (Fernandez-Jover et al. 2008; Ballester-Moltó et al. 2015).

1.4 Positive and negative fish farming impacts on wild fish

The effects of fish farming on wild fish populations can be positive, negative, a combination of both or none. Wild fish feeding on high energy feed can lead to improved Fulton's condition indices (FCI) and hepatosomatic indices (HSI), increased lipid levels, and modified fatty acid (FA) profiles (e.g. Skog et al. 2003; Arechavala-Lopez et al. 2011; Dempster et al. 2011; Izquierdo-Gómez et al. 2015). Increased fat content, particularly in gadoids, has been linked to increase in egg production (Marshall et al. 1999). However, wild fish feeding on waste feed can have a modified FA profile which may reduce reproductive performance in terms of egg quality and larval survival (Izquierdo et al. 2001). It is unclear what the long term physiological consequences are in wild fish that have been influenced by fish farming.

Fernandez-Jover et al. (2009) reported that a number of juvenile fish used coastal fish farms in the Mediterranean Sea as habitats for settlement possibly for protection from predators which in turn can improve survival rates. The fatty acid (FA) profile of the juvenile fish was modified as a result of the consumption of zooplankton which was influenced by particulate organic matter (waste feed and faeces from farmed fish) and the dissolved nutrients (Fernandez-Jover et al. 2009). It is not known whether modified FA profiles affects the physiology of juvenile fish around fish farms (Fernandez-Jover et al. 2009). Similarly, Fernandez-Jover and Sanchez-Jerez (2015) reported that coastal sea cages provide new settlement habitat for a number of larval and juvenile fish around fish farms in the Mediterranean Sea. Furthermore, the researchers, using otolith shape analysis, noted that growth of wild fish is affected possibly because of feeding on waste feed. Abaad et al. (2016) reported changes in somatic (body shape) and otolith growths in wild fish consuming waste feed around fish farms in the Canary Islands. Faster growth in younger individuals could lead to earlier maturation which can have population level impacts and changes in otolith structure can have implications on the sensitivity of the inner ear of fish (Abaad et al. 2016 and references therein).

Fish farming can lead to potential negative effects such as the transfer of diseases and parasites between farmed and wild fish (see subsection 1.7.3). Dempster et al. (2011) reported elevated levels of external parasites such as sea lice and lower levels of internal parasites (*Anisakis* simplex) in wild fish sampled near fish farms in Norway. Elevated levels of mobile sea lice in farm associated fish occurred because of the direct transfer between wild and farmed fish. On the other hand, the decrease in internal parasites in wild fish was related to the increased consumption of parasite free waste feed which decreases the consumption of parasite hosts (e.g. small fish and crustaceans) (Dempster et al. 2011). Dempster et al. (2011) concluded that although there were some alterations in the parasite loads of wild fish around fish farms, the fish benefitted more from the extra food provided by the waste feed which was evident in improved condition.

Fish farms provide easily available food resources that delay the offshore migration of saithe (Otterå and Skilbrei 2014). Otterå and Skilbrei (2014) using acoustic tags studied the movement patterns of saithe near fish farms in Norway. Additionally, the researchers used external T-bars tags to follow the long-distance migration patterns of saithe. Otterå and Skilbrei (2014) concluded that fish farming has an effect on the migration patterns of saithe in such a way that a substantial part of the population is not migrating, and offshore migration occurs at larger sizes.

Some ecological effects of fish farming are less easy to quantify. Papastamatiou et al. (2010) using acoustic telemetry reported that sandbar shark (*Carcharhinus plumbeus*) consistently returned to open ocean Hawaiian fish farms whereas the tiger shark (*Galeocerdo cuvier*) only visited the fish farms for short periods. The researchers noted the attraction of predators by fish farms means that predators are removed from somewhere else which can result in trophic cascades (Papastamatiou et al. 2010).

Uglem et al. (2014) noted that the overall ecological impacts of aquaculture activities and wild fish are complex and vary depending on a number of factors such as species, sexes, seasons, years, ontogenetic stages, locations and other factors such as implications for stakeholders (e.g. fishing activities, fish farming).

1.5 Attraction, ecological trap or production sites?

The potential of artificial or natural objects in marine environments to attract and/or aggregate fishes is well established (Pickering and Whitmarsh 1997; Sanchez-Jerez et al. 2011). Some structures such as artificial reefs have been deployed to enhance commercial

and recreational fisheries based on the assumption that new habitat is provided which leads to new fish biomass production (Bohnsack 1989). However, fish could simply be attracted to marine structures from nearby areas rather than enhance local production. This is the 'attraction-production' debate that has dominated marine reef literature for the past few decades (Lindberg 1997; Pickering and Whitmarsh 1997). Based on the attraction hypothesis fish move to the vicinities of artificial structures and aggregate around the structures with no increase in production. On the other hand, based on the production hypothesis, the artificial structures can maximise production; fish settle, grow and contribute to the population in terms of biomass (Pickering and Whitmarsh 1997). Reubens et al. (2014) noted that considering only the continuum of attraction and production argument is not a true representation of reality and thus suggested that ecological traps need to be added to the argument.

Ecological traps are artificial habitats that are of poor quality but are chosen by animals over other habitats that are of better quality leading to reduced survival and/or reproductive performance (reviewed by Battin 2004). Animals use cues (e.g. olfactory, auditory, and visual) shaped by natural selection to select habitats that would maximize their survival/reproduction (Schlaepfer et al. 2002). Ecological traps often occur in environments where human-driven change is much faster than the natural change leading to the uncoupling of cues that animals use to select for high quality habitats (Schlaepfer et al. 2002). For example, insects can be deceived and attracted to the polarized light from asphalt surfaces (e.g. roads) which mimics highly polarized water surface. The insects lay their eggs on the asphalt rather than nearby water bodies which would lead to perishing of the eggs because of dehydration (Kriska et al. 1998). Sea turtles hatchlings rely on natural visual cues to journey from their nest to the ocean during the night. However, when turtles are exposed to artificial light at night their movement towards their suitable ocean habitat is disrupted (Tuxbury and Salmon 2005).

It is worth noting that animals can potentially adapt to an ecological trap or cease to exist unless the trap is removed before the population goes extinct (Battin 2004). Although species response to environmental change is not always easy to predict, animals can adapt successfully to an environmental change depending on the learning capacity of the animal, availability of different habitats, and most likely when the rate of environmental change is modest (Battin 2004). Animals such as birds and mammals that

have higher cognitive ability are less likely to be trapped than insects (Hale and Swearer 2016). The cognitive ability of fish should be somewhere in between that of birds and insects.

Animals that successfully use cues based on their evolutionary past to respond to environmental change are less vulnerable to ecological traps (Battin 2004). If the new cues are very different from those of their evolutionary past then selection will favour animal that can respond to the new cues (Sih et al. 2011). Additionally, animal that can adapt to new environmental changes have also gained traits from their evolutionary past which allows them to respond to change and persist in the long term (Sih et al. 2011).

Maladaptive decisions are not restricted only to habitat selection but more broadly to any maladaptive behavioural decision (e.g. migration time, reproduction time, food quality etc.) that occur because of human-driven changes. Evolutionary trap is the term used to describe these broader maladaptive behavioural decisions in anthropogenically altered environments (Schlaepfer et al. 2002).

Demonstrating the presence of an ecological trap is not always easy. According to Robertson and Hutto (2006) for an ecological trap to exist three conditions need to be met: 1) animals select one habitat over another habitat, 2) survival rate and/or reproductive performance of the animal differ between both habitats, 3) the animal has poorer survival and/or breeding performance as a result of exploiting the new habitat. On the other hand, if the exploitation of a habitat results in improved survival and/or reproductive performance of an animal then the habitat can potentially act as a population source (Dempster et al. 2011). Coastal sea cages, like other artificial structures, have the potential to act as a population source or an ecological trap depending on the impacts generated by the farms (Dempster et al. 2011; Uglem et al. 2014).

In theory, coastal fish farms can benefit wild fish communities attracted to the cages. Wild fish consuming high energy artificial waste feed from fish farms may lead to changes in physiological processes such as rapid growth and enhanced reproduction (Uglem et al. 2014). Production in local fisheries could, potentially, be increased by the spill-over of adult fish from fish farms and an increase in spawning stock biomass which would boost recruitment (Dempster et al. 2002; see Özgül and Angel 2013 and references therein). Dempster et al. (2002) suggested that coastal sea cages can act as small marine protected areas by excluding fishing effort around the fish farms. Similarly, Özgül and Angel (2013) suggested that fish farms in the Red Sea may act as small marine protected areas which can be of benefit to touristic activities (e.g. eco-tourism). If fish farms attract

species that are endangered then spatial protection can be of benefit to these species (Dempster et al. 2002; Özgül and Angel 2013).

However, coastal fish farms might negatively impact the wild fish populations attracted to their vicinities by the transmission of pathogens,potential exposure to contaminants when feeding on waste feed, and increased predation(Skog et al. 2003; Otterå et al. 2009; Uglem et al. 2014). Additionally, if the wild fish around the fish farms are exploited by various fishing activities it can have negative impacts on the fish populations (Fernandez-Jover et al. 2008; Arechavala-Lopez et al. 2011).

If the overall impacts of fish farming on wild fish populations are negative, then fish farms can be ecological traps but if the overall impacts are positive then fish farms can be population sources. Dempster et al. (2011) evaluated whether salmon farms in Norway act as ecological traps or population sources for wild saithe and cod. The researchers found that the fish farms provided an additional food resource for wild fish and this was evident in the higher somatic and liver condition indices in fish sampled near fish farms as compared to fish sampled from areas with no fish farms. On the other hand, increase in external parasites and decrease in internal parasites was found for wild fish near farms as compared to those away from farms (Dempster et al. 2011). Dempster et al. (2011) concluded that although positive (improved condition) and negative effects (parasite alterations) were found for wild fish near fish farms the overall benefits outweighed the negative and therefore fish farms are population sources rather than ecological traps for the studied species.

1.6 Marine fish farming and impacts on commercially targeted species

Coastal fish production can affect wild fish around sea cages that are of commercial importance at the individual level in a positive (e.g. improved condition) or negative way (e.g. exposure to contaminants) with subsequent impacts on the commercial and recreational fisheries (see Uglem et al. 2014).

1.6.1 Impacts on local commercial and recreational fisheries

Coastal fish farms can attract large number of wild fish that are of commercial and recreational interests (reviewed by Uglem et al. 2014). For example, bogue (*Boops boops*) and saithe are often found around fish farms and both species are of high economic importance to the fishing industries in the Mediterranean Sea and Norway, respectively

(Arechavala-Lopez et al. 2011, 2015a; Dempster et al. 2009). Tuna farms in the Adriatic Sea attract wild tuna (*Thunnus thynnus*) which is targeted by local fisherman (Šegvić Bubić et al. 2011). Other species of commercial importance that are attracted to tuna farms include species belonging to the families of Sparidae, Carangidae, and Scombridae (Šegvić Bubić et al. 2011). Fernandez-Jover et al. (2008) reported a number of fish species belonging mainly to families of Clupeidae, Sparidae, Mugilidae, and Carangidae near fish farms in the Mediterranean Sea. The authors noted that many of these species are targeted by local fishers which may affect the distribution of these populations on a regional scale. Similarly, Dr. Tom Wilding from SAMS noted that intensive aquaculture is expected to change the distribution of some species.

Wild fish near fish farms feeding on waste feed can increase in fish biomass and condition which can lead to a localised increase in fisheries biomass (Machias et al. 2005, 2006; Arechavala-Lopez et al. 2011; Uglem et al. 2014). Arechavala-Lopez et al. (2010) using acoustic tagging reported that fish farms and fishing grounds in the Mediterranean Sea are connected via the movement of commercially important fish. Furthermore, Arechavala-Lopez et al. (2011) reported that wild fish influenced by fish farms make a significant catch in artisanal fisheries in the Mediterranean Sea. Uglem et al. (2014) noted that most fish attracted to fish farms in Norway are from local fishing grounds. In Norway, wild fish aggregations around salmon farms are not accessible to commercial fisheries within 100 m from the perimeter buoys of the farm (see Uglem et al. 2014) which can be considered as mini MPAs (Dr. Tom Wilding, SAMS, pers. comm., January, 2017).

Local fisheries can be affected by fish farms directly through the alterations in the dispersal of commercially important wild fish that are attracted to fish farms and indirectly through changes in reproductive performance of the fish (Uglem et al. 2014). Dempster et al. (2011) reported greater gonad mass of wild cod caught near salmon farms in Norway as compared to cod caught from areas with no fish farming. Although, reproductive performance of wild fish near fish farms can potentially be improved modification in fatty acid profiles may not be optimal for egg and larval quality (Fernandez-Jover et al. 2011a; Uglem et al. 2014). Abundant food resources around fish farms can boost growth performance of wild fish leading to an earlier age of sexual maturation and subsequent changes in spawning migrations (Otterå and Skillbrei 2014; Uglem et al. 2014). There is lack of information on how potential fish farming impacts

on reproduction of wild fish may affect the local population dynamics (see Uglem et al. 2014).

Less information is available on the interactions between fish farms and recreational fishing activities (Uglem et al. 2014). Uglem et al. (2014) suggested that recreational fishing can be affected by fish farms in a similar way that commercial fishing is affected.

1.6.1.1 Effects on flesh quality of wild fish for human consumption

Wild fish consuming on waste feed from fish farms can be affected by changes in flesh quality (e.g. increased softness, high occurrence of gaping, abnormal coloration and unusual smell) that can be unacceptable by customers and thus affect sales of the local fisheries (see Skog et al. 2003; Otterå et al. 2009; Uglem et al. 2014). Additionally, fish that are fattier spoil more rapidly than lean fish and thus increased lipid levels in wild fish consuming high energy pellets can decrease their shelf life (Bogdanović et al. 2012).

It is also worth noting that methods of capturing fish near and away from cages could have an impact on the stress of the fish with consequent impacts on the flesh quanlity. Toledo-Guedes et al. (2016) reported that saithe sampled using commercial gillnets were found to be more stressed than saithe sampled using jigging. However, the capture method did not seem to show obvious differences in the flesh quality of the fish (Toledo-Guedes et al. 2016).

1.6.1.2 Chemicals and wild fish

Production of fish in sea cages can introduce a number of chemicals such as medicinal substances, heavy metals and contaminants into the marine environment that can be consumed by wild fish directly through the feed or indirectly through other natural prey that have consumed these chemicals (Uglem et al. 2014).

Bustnes et al. (2010) reported that salmon farms in Norway increased the levels of lipid-soluble persistent organic pollutants in wild fish near the sea cages. In another study by Bustnes et al. (2011) 30 elements, including mercury (Hg), were evaluated in the livers of cod and saithe near fish farms and no overall increase in harmful elements was detected. An earlier study by deBruyn et al. (2006) reported elevated levels of Hg in long-lived demersal rockfish (*Sebastes* sp.) caught near salmon farms as compared to fish caught from areas with no farming activity in British Columbia, Canada. The authors

suggested that there are two possible routes for rockfish to bio-accumulate Hg. First route of bio-accumulation of Hg is a result of mercury loading in fish faeces and waste feed and the second route is via the mercury present (native and added) in the sediment. The organic input from fish farms leads to sediment anoxia directly beneath the sea cages which can make Hg more bio-available through the biomethylation to benthic prey which in turn are consumed by rockfish (deBruyn et al. 2006).

1.7 Fish farming industry and wild fish aggregations: benefits and costs

Wild fish around fish farms can be of benefit to the farming industry but can also pose risks in terms of increased numbers of predators around the sea cages and also the potential risk of pathogen transmission between wild and cultured species (reviewed by Sanchez-Jerez et al. 2011; Uglem et al. 2014).

1.7.1 Wild fish can mitigate unwanted fish farming effects

Uglem et al. (2014) reviewed the effects of wild fish attracted by sea cages on farming practices in Norway and noted that the consumption of waste feed by wild fish reduces potential negative impacts on the benthos. Moreover, wild fish can recapture escaped farmed fish (see Uglem et al. 2014). In two experimental setups, it was found that wild fish aggregations consumed 40-80% of the waste feed produced by fish farms (Vita et al. 2004; Felsing et al. 2005). However, Ballester-Moltó et al. (2017a), reported that wild fish consumed about 18% of the particulate wastes released by fish farms. The differences between these studies (Vita et al. 2004; Felsing et al. 2005; Ballester-Moltó et al. 2017a) in the amount of waste feed consumed by wild fishes may be caused by differences in methodologies, farm operating conditons, environmental conditions etc. (Ballester-Moltó et al. 2017ab).

Wild fish around sea cages consume waste feed that is transformed into wastes through excretion and defecation which can affect the distribution of nutrients in the environment (Fernandez-Jover et al. 2007b). Fernandez-Jover et al. (2007b) reported that rapid leaching of nutrient from faeces of wild fish around fish farms reduces the organic impact on the sediment. The additional nutrients released into the water column can be a source of food for phytoplankton and bacteria (Fernandez-Jover et al. 2007b). Settlement times of faeces of wild fish are also slower than waste feed which allow the distribution of nutrients over a wider area (Fernandez-Jover et al. 2007b).

Feeding habits of some wild fish species may reduce the fish farming impacts on the seabed. For example, Katz et al. (2002) reported that bioturbation by bottom-feeding grey mullet (*Mugil cephalus*) can reduce the anoxic conditions in organically enriched sediments under fish farms.

Escapes of fish (juvenile and adult) from sea-cage aquaculture are often reported amongst most farmed fish species worldwide (reviewed by Jensen et al. 2010). Wild fish around fish farms can reduce the number of escaped small fish by preying on them (see Uglem et al. 2014 and references therein). Serra-Llinares et al. (2013) studied the dispersal patterns of juvenile cod released from a Norwegian farm and reported high predation rates by large wild fish aggregating around the cages.

1.7.2 Coastal fish farms attract predators

The presence of farmed fish and wild fish aggregations around the sea cages attracts a large range of predators (e.g. harbour seals (*Phoca vitulina*), grey seals (*Halichoerus grypus*), cormorants (*Phalacrocorax carbo*), shags (*Phalacrocorax aristotelis*), herons (*Ardea cinerea*), otters (*Lutra lutra*) and mink (*Mustela vison*)) which can have negative impacts on the fish farming industry (Quick et al. 2004; Díaz López and Bernal Shirai 2007 and references therein). Fish farming can have increased monetary loss as a result of predators damaging the nets which can lead to losses in stocks and feed. Moreover, predators can also increase the transmission of pathogens (reviewed in Sanchez-Jerez et al. 2011; Arechavala-Lopez et al. 2015b). Predators such as the bluefish (*Pomatomus saltatrix* (L.)) are of concern in the Meditteranean Sea because they enter the cages and prey on the cultured fish leading to economic losses (Sanchez-Jerez et al. 2008). In their review, Uglem et al. (2014) did not find any similar reports in Norway but noted that some predators such as the spiny dogfish (*Squalus acanthias*) were suspected in making holes in nets to prey on cultured species and causing escapees from the farms.

1.7.3 Pathogen transmission and wild fish

Fish in marine aquaculture facilities are often held at very high densities for long periods of time in the same location which facilitates the movement of pathogens between farmed and wild fish (Johansen et al. 2011). Transmission of pathogens can take place from farmed to wild fish and vice versa but there is insufficient information on the potential risk of wild fish to transmit pathogens (Uglem et al. 2014). Dempster et al.

(2009) noted that wild fish species such as saithe, cod, haddock and Atlantic mackerel (*Scomber scombrus*), often found around Norwegian salmon farms, can act as vectors for pathogens and parasites for farmed fish. For example, saithe may act as a natural reservoir of the salmonid alphavirus (SAV) (causative agent for pancreas disease in salmon) (Graham et al. 2006) and is a carrier of infectious pancreatic necrosis virus (IPNV) (causative agent for infectious pancreatic necrosis in salmon) (Wallace et al. 2008) (see also Johansen et al. 2011).

Uglem et al. (2014) pointed out that for wild fish to transfer pathogens from farmed fish to other farms or wild fish populations three assumptions need to be met; wild fish around fish farms need to stay around long enough for the pathogen transfer to occur, wild fish need to move frequently to other farms and locations, and the same pathogens are shared between farmed and wild fish. A number of reports provide evidence for the first two assumptions that wild fish (e.g. saithe and cod in Norway and mullets (*Liza aurata* and *Chelon labrosus*) and bluefish in the Mediterranean) around fish farms can be resident for several months and move among farms but less is known of the third assumption (Uglem et al. 2008, 2009, 2014; Dempster et al. 2010; Arechavala-Lopez et al. 2010, 2013; Sanchez-Jerez et al. 2011; Otterå and Skilbrei 2014).

Fernandez-Jover et al. (2010) noted that the overall parasite communities in wild fish sampled near sea cages in the Meditteranean Sea were not affected by the presence of the farms. The authors also noted that the presence of fish farming may increase or decrease the number of parasites depending on the species. McGeorge and Sommerville (1996) reported that although there were more parasites on wild fish around Scottish fish farms than on the cultured fish there was no indication of interactions between parasites in farmed and wild fish.

Johansen et al. (2011) reviewed the possible role of wild fish as vectors for pathogens and concluded that there is limited research and more studies need to be conducted to understand the interactions of pathogens between wild fish and farmed fish.

1.8 Capture fisheries and coastal aquaculture in Scotland

The majority of the studies related to marine fish communities and interactions with aquaculture activities were conducted in the Mediterranean Sea and Norway. Little is known about the ecological impacts of aquaculture activities on native marine fish communities in Scotland.

Because of the high productive marine waters in Scotland both commercial and aquaculture activities are present (Baxter et al. 2011). In this section, I focus on the main species of interest in commercial fisheries and aquaculture activities in Scotland.

1.8.1 Capture fisheries

In 2014, a total of 481,000 tonnes of marine fish (pelagic and demersal) and shellfish were landed by Scottish vessels and the total landed in Scotland (Northern North Sea and the West Coast) was 375,149 tonnes (Scottish Government 2015). The majority of the captured fish were pelagic (54%) which included mackerel (149,325 tonnes) and herring (*Clupea harengus*) (39,458 tonnes). Demersal fish (32% of the total catch) included mainly haddock (35,806 tonnes), saithe (17,374 tonnes), hake (*Merluccius merluccius*) (14,594 tonnes), cod (13,486 tonnes), whiting (9,613 tonnes), monkfish (8,632 tonnes) and ling (*Molva molva*) (5,545 tonnes). Shellfish accounted 14% of the total catch and included nephrops (*Nephrops norvegicus*) (20,171 tonnes), edible crabs (12,365 tonnes) and scallops (10,629 tonnes).

The main areas in Scotland for pelagic, demersal and shellfish fisheries are Peterhead (east coast), Shetland (north), and Fraserburgh (east coast). Shellfish are mainly landed in the south-west and south-east coasts of Scotland. Landings in the north-west coast are dominated by demersal species and to some extent shellfish (Scottish Government 2015).

1.8.2 Aquaculture

Aquaculture (fish and shellfish) production plays a significant role in the Scottish economy in creating employment opportunities and developing rural areas (Scottish Government 2014). The industry is growing and aiming to reach a total marine fish production of 210,000 tonnes by 2020 (Scottish Government 2014). The main species of interest to the industry is the Atlantic salmon. Production of salmon at a commercial level started in the late 1960s and has grown continuously (Ellis et al. 2016). Worldwide Scottish salmon production is third after Norway and Chile (Scottish Government 2014).

Marine fish production amounted to 181,000 tonnes in 2014 with main species Atlantic salmon (179,000 tonnes) followed by sea grown rainbow trout (*Oncorhynchus mykiss*) (2,000 tonnes) and a smaller total production (120 tonnes) of other species such as Artic charr (*Salvelinus alpinus*), brown/sea trout (*Salmo trutta*), cod, halibut

(*Hippoglossus hippoglossus*), lumpsucker (*Cyclopterus lumpus*) and several species of wrasse (Labridae) (Munro and Wallace 2015a). Lumpsucker and wrasse species are produced as cleaner fish for the salmon industry (Munro and Wallace 2015a).

The production of shellfish is much lower than the fish farming production in Scotland. Shellfish production in 2014 was mainly dominated by mussels (*Mytilus* spp.) (7,700 tonnes) and Pacific oyster (*Crassostrea gigas*) (271 tonnes). Other species with lower production included native oyster (*Ostrea edulis*) (19 tonnes), queen scallop (*Aequipecten opercularis*) (1 tonne) and scallop (*Pecten maximus*) (6 tonnes) (Munro and Wallace 2015b). The industry is planning to reach shellfish production of 13,000 tonnes by 2020 (Scottish Government 2014). The main locations for the marine aquaculture activities are on the West Coast of Scotland, along with Western Isles, Orkney and Shetland Isle (Mente et al. 2008; Munro and Wallace 2015b).

Mariculture in Scotland faces similar environmental challenges as in other countries; issues with pathogens, escapees from fish farms, local and wider scale of eutrophication, changes in benthic biodiversity beneath sea cages and environmental impacts because of waste feed (e.g. Mente et al. 2006; Price et al. 2015). There has been an increased interest in evaluating the ecological impacts of aquaculture activities on marine fish communities in a number of countries (see subsection 1.2.1 of this chapter). However, little is known on the ecological impacts of aquaculture activities on the wild fish communities on the West Coast of Scotland (e.g. Carss 1990, 1996; Mente et al. 2008).

The West Coast of Scotland is characterised by numerous sea lochs which are glacially overdeepened valleys and can be considered specialised estuaries (Edwards and Griffiths 1996). Many of the sea lochs are sheltered and suitable for aquaculture activities (Mente et al. 2008; Munro and Wallace 2015ab). A number of commercially important juvenile gadoids such as haddock, cod, and whiting also use sea lochs as nursery grounds (Ware 2009). Other commercially important migratory species such as mackerel use the West coast as feeding grounds during the summer months (Lockwood 1988). Young gadoids (e.g. saithe and cod) and migratory species such as mackerel have been noted around fish farms on the West Coast of Scotland (Carss 1990; personal observation by Pearson and Black 2001). Dr. Tom Wilding from SAMS has consistently observed and caught mackerel near fish farms on the West coast of Scotland (pers. comm., January 2017).

As both commercial and recreational fisheries and aquaculture activities are present on the West Coast it is expected that there will be ecological interactions between the two sectors in terms of marine fish communities. Additionally, the aquaculture industry is expected to increase its production which raises the need to assess the ecological impacts of aquaculture activities on marine fish populations at the individual, population and ecosystem levels. This is needed in order to evaluate the sustainability of the sector in terms of ecological interactions (Mente et al. 2008; Scottish Government 2014).

In this thesis, I focus on the ecological interactions between aquaculture activities and three fish species of commercial interest: mackerel, whiting and saithe.

1.9 Fish species studied in this thesis

Atlantic mackerel (Figure 1.1A) is an abundant and economically valuable pelagic species distributed on both sides of the Atlantic Ocean (Jansen 2014; Trenkel et al. 2014). In the Northeast Atlantic, mackerel ranges from Morocco to Norway. Summer migrations of mackerel expand the range from Greenland to the Western Baltic Sea. Mackerel has also been observed in the Meditteranean Sea in the south, Skagerrak, Kattegat and the westernmost Baltic Sea (Jansen 2014; Trenkel et al. 2014). In the Northwest, it ranges from the Gulf of Maine to the Gulf of St. Lawrence (Trenkel et al. 2014). Atlantic mackerel performs annual migrations between spawning and feeding grounds. In the Northeast Atlantic, mackerel spawn in March along the shelf break from Spanish and Portuguese waters to the West Coast of Scotland and in June in the North Sea (see Trenkel et al. 2014). Following spawning mackerel are dispersed into adjacent waters and northwards to feed (Trenkel et al. 2014). Mackerel matures on average around 3 years of age (Lockwood 1988). Mackerel can reach 60 cm in length and over 20 years of age. The diet of mackerel includes zooplankton, larvae and small fish (Langøy et al. 2006, 2012; Skaret et al. 2015). Mackerel are planktivorous fish that feed through filter and particulate modes of feeding or both and the choice of feeding strategy depends on the size and abundance of the prey (Pepin et al. 1988; Langøy et al. 2006). In more coastal areas mackerel appears to consume more fish whereas in the opean ocean zooplankton dominates the diet (see Skaret et al. 2015 and references therein). Based on the diet composition the trophic level of adult mackerel is 3.63[1].

[1] www.fishbase.org [Accessed: 13 May 2018].

Whiting (Figure 1.1B) is an abundant benthopelagic gadoid species distributed across the Northeast Atlantic from the Barents Sea to the North Sea; from Iceland to Portugal (Whitehead et al. 1986). It is also found in the north coast of the western Mediterranean Sea and the Black Sea, adjacent areas of the Adriatic Sea, Aegean Sea, Sea of Marmara, and Azov Sea (Whitehead et al. 1986). Whiting have extended spawning season from February to June across the range of their distribution (see Bailey et al. 2011). Whiting have extended pelagic phase which is longer than that in other common gadoids; following a 6 month pelagic phase the juveniles move to shallow inshore waters remaining there for another 6 months (see Bailey et al. 2011). By the age of 2 most whiting are mature and can spawn (see Bailey et al. 2011). Whiting are opportunistic feeders mainly preying on fish and crustaceans (Hislop et al. 1991). Based on the diet composition the trophic level of adult whiting is 4.36[1]

Saithe (Figure 1.1C) is a benthopelagic species that occurs on both sides of the North Atlantic; in the eastern Atlantic from the coasts of Bay of Biscay to the Barents Sea across Greenland, Iceland, Faroe Islands, Spitzbergen and Novaya Zemlya (young), Skagerrak, Kattegat, Bay of Mecklenburg (rare), Kola peninsula, White Sea (occasionally) and rarely reported in the Baltic Sea (Harms 1993; Byrkjedal and Høines 2007; Rolbiecki et al. 2008). In the Western Atlantic, saithe is found at the border between the USA and Canada with highest abundance on the western Scotian shelf, Georges Bank, and in the Gulf of Maine (Svetovidov 1986; Olsen et al. 2010). Northeast Atlantic saithe spawns offshore during winter followed by recruitment to coastal areas for a period of 2-4 years and then move to deeper offshore waters as they mature (see Armannsson et al. 2007). Saithe is often found throughout the water column where they form shoals when feeding. Saithe is an opportunistic feeder mainly preying on fish and crustaceans which also vary with habitats and seasons (Wheeler 1978; Svetovidov 1986; Tyrrell et al. 2007). The trophic level of adult saithe is estimated to be 4.31[1].

Figure 1.1 Atlantic mackerel (*Scomber scombrus*) (Linnaeus 1758), (A), Whiting (*Merlangius merlangus*) (Linnaeus 1758) (B), Saithe (*Pollachius virens*) (Linnaeus 1758) (C).

1.10 Objectives and structure of the PhD research

The overall objective of this research was to examine the direct and indirect ecological interactions between aquaculture activities and wild marine fish communities in Scotland. The impacts of two fish farms on wild fish communities were evaluated at the individual, population and ecosystem levels. Accordingly, the thesis is split into three parts. Part I (Chapters 4, 5, 6) of this thesis includes empirical studies evaluating the direct ecological impacts of two fish farms on mackerel, whiting and saithe at the individual level. Part II (Chapters 7 and 8) includes modelling studies evaluating the ecological impacts of fish farming at the population and ecosystem levels. Indirect ecological impacts of both fish and mussel farming on wild fish communities in a sea loch are included in Chapter 8. Part III (Chapter 9) includes a general discussion and conclusions.

In Chapter 2, I give a general background information on the fieldwork and laboratory approaches (stomach content and fatty acid analysis) followed by modelling approaches (statistical, single-species and ecosystem models) used in this research.

In Chapter 3, I give detailed description of location sites (farm and reference sites), underwater video recordings, macrobenthic and fish sampling methodologies, stomach content analysis, total lipid and fatty acid analysis.

In 2013, I conducted a study near one farm and a corresponding reference site and collected mackerel and saithe. The aim of the study was to test various empirical techniques and to collect the most abundant species around the cages and to determine the dietary composition of the fish. Fatty acid analysis was used to detect whether the fish have consumed waste feed. The effect of the farm on the biological condition of the mackerel and saithe was also evaluated (Chapter 4).

To confirm the results of 2013, the study was extended in 2014 (Chapter 5) by including a second farm and including additional reference sites for the sampled fish. The fish sampling size was also increased. The most common fish species caught around both fish farms were mackerel and whiting. The same analysis (stomach content, fatty acids and condition) as in Chapter 4 was performed on both mackerel and whiting (Chapter 5).

In Chapter 6, I combined the data from fieldwork conducted in 2013 and 2014 for all three species (mackerel, whiting and saithe) in order to explore whether coastal cages act as ecological traps or productivity sites for the selected species. To answer the question, I used proxies of fitness such as diet, length and biological condition for each

species caught near the cages and compared it to diet, length and condition of fish caught away from cages.

Results of Chapter 6 indicated that coastal sea cages act as population sources and not ecological traps for mackerel, whiting and saithe. The results were based on limited data and using data for individual fish. Therefore, in Chapter 7 I explored whether sea cages act as ecological traps or population sources for mackerel and whiting at the population level. I built a phase space model to explore hypothetical combinations of positive (e.g. increase in fecundity) and negative (e.g. increase in mortality) effects of sea cage farming on the mackerel and whiting populations.

In Chapter 7, I used a single species modelling approach and did not account for any trophic interactions. Therefore, an ecosystem model for a sea loch was built in Chapter 8 to account for trophic interactions and quantify trophic flows to address effects of aquaculture activities (fish and mussel farming) on a particular species that interacts with other species, principally because changes in the abundance of one component of the ecosystem will change the constraints on other parts of the ecosystem.

In Chapter 9, I provide an overview of the knowledge obtained during this four-year PhD research. The conclusions of all chapters are discussed into a broader perspective in relation to ecological processes and fishery activities. The use of empirical and modelling approaches as a combined method to understand the wider context is also discussed.

CHAPTER 2
EMPIRICAL AND MODELLING APPROACHES TO EVALUATE AQUACULTURE EFFECTS ON WILD FISH POPULATIONS

In this chapter, I give background information on the main empirical and modelling approaches used in this thesis to study the ecological interactions between aquaculture activities and wild fish communities at the individual, population and ecosystem levels. Empirical studies (Chapters 4-6) were used to assess the direct impact of artificial waste feed on the physiology of wild fish caught using rod and line around two fish farms. A single-species modelling approach was used to evaluate the direct effects of fish farming impacts at the population level of wild fish communities (Chapter 7). Species exist in complex systems and direct effects of fish and mussel farming can transmit indirect effects throughout the food web. Ecosystem-based modelling was used to evaluate direct and indirect effects of fish and mussel farming on wild fish communities (Chapter 8).

Background information on the fieldwork and laboratory approaches are described in section 2.1 and the modelling approaches in section 2.2 of this chapter.

2.1 Empirical (fieldwork and laboratory) approaches

Coastal fish farming releases large amounts of organic by-products such as particulate matter (uneaten food, faeces) and dissolved metabolic waste (ammonia) (reviewed by Holmer 2010; Uglem et al. 2014; Price et al. 2015). The addition of these food resources into the environment can impact the diet, condition, fat and FA profiles of wild fish in the vicinities of coastal fish farms (Dempster and Sanchez-Jerez 2008).

In this section, I give background information on the main methods I used to collect data from the field to detect aquaculture impacts on the diet, biological condition, and changes in total lipid and FA composition of wild marine fishes caught using rod and line in the vicinities of two fish farms. Description of fieldwork (subsection 2.1.1 and Chapter 3) and laboratory methods (stomach content analysis (subsection 2.1.2), condition (subsection 2.1.3), lipid and FA analysis (subsection 2.1.4)) used in this thesis are described in the following subsections. In Chapter 3, I give more details on the sampling procedures, locations and laboratory analysis of samples collected during fieldwork.

2.1.1 Fieldwork methodologies

In this section, I briefly give an overview of some important factors that need to be taken into consideration in the sampling design related to studying the impacts of fish farming on wild fish communities.

2.1.1.1 Overview of ecological experimentation

To test what impacts coastal aquaculture activities have on wild fish aggregations there needs to be clear hypothesis(es) and a sampling design that will have replication (measuring variability) and ensure results are not confounded (Underwood 1997; Kingsford 1999). Collecting data with no clear research goals will result in data that is useless (Underwood 1997). As the environment and habitat varies in times and space there needs to be a carefully planned sampling design to consider spatial and temporal variability and interactions between space and time, and logistics (Underwood 1997, 2009). Spatial variation includes differences of ecological processes in different places and temporal variation includes differences in biological processes related to seasonality, different ages or stages of development of an organism (Underwood 1997).

Coastal aquaculture activities have an impact on wild fish populations (see Chapter 1) which should be taken into consideration in the sampling design (see Kingsford 1999). To evaluate an impact there needs to be comparative studies. The simplest sampling design in detecting an impact on the environment is to collect data before and after an impact (Green 1979). BACI (Before, After, Control, Impact) design is common in assessing anthropogenic impacts on the environment (Underwood 1992; Kingsford 1999). As there is spatial and temporal variability in abundances in marine organisms between different locations it is a requirement in impact studies to compare the impact site(s) with several control (or reference) sites (Kingsford 1999). Different BACI designs have been developed to detect anthropogenic impacts in the environment. For example, beyond-BACI design allows an impact site to be compared to several control sites including before and after the impact (Underwood 1992; Kingsford 1999). A further development to the BACI design is the M-BACI (multiple before/after control/impact) design which takes into account several impacted sites and compares them to several control sites including also before and after impacts (Kingsford 1999). A number of studies have used before/after and/or control/impact designs to detect coastal aquaculture

impacts on wild fish populations (e.g. Tuya et al. 2006; Dempster et al. 2009, 2011; Tanner and Williams 2015).

The sampling design of a study should take into account the methods used to catch or count fish for the estimation of abundances (Kingsford 1999). Methods for sampling fish near artificial structures include non-destructive methods such as underwater visual censuses (e.g. direct observation by divers) and extractive methods such as hook and line, gill netting, seine netting (see Kingsford 1999; Lowry et al. 2012). Capture methods are often destructive and thus there is a widespread use of visual census techniques to observe fish around artificial structures (e.g. Lowry et al. 2012). Scuba (Self Contained Underwater Breathing Apparatus) diving is a relatively rapid, non-destructive method to observe fish and allows a number of variables to be measured such as number of fish and habitat characteristics (Lowry et al. 2012). However, diver-based techniques are restricted by depth, temperature, time, health safety issues (e.g. shark attacks in Australia) and can affect the behaviour of fish in response to divers (Tanner and Williams 2015). Alternatively, underwater video techniques which are not restricted by the physical limitations of divers, avoid the change in behaviour of fish that can be induced by the presence of divers, and provide information on habitat and species behaviour (see Tanner and Williams 2015 and references therein). All sampling techniques have advantages and disadvantages and depend on the research question, fish of interest, environmental conditions and habitat (Tanner and Williams 2015). Lowry et al. (2012) recommended the use of multi-method approach such as the use of diver techniques and underwater video techniques.

To count fish around coastal fish farms, previous studies have used various methods including non-destructive techniques such as diver-based techniques (Mediterranean Sea, Dempster et al. 2002; Canary Islands, Tuya et al. 2006), underwater video camera (Norway, Dempster et al. 2009), and baited remote underwater video (Australia, Tanner and Williams 2015).

Another factor to consider when choosing control sites to detect the impacts of artificial structures on wild fish populations is the spatial extent of the impact on the fishes (Kingsford 1999). Dempster et al. (2010) evaluated the spatial distribution of wild fish around salmon farms in Norway. The researchers reported highest fish abundance near the sea cages and the aggregation patterns of fish near the sea cages depended on the species.

Most marine organisms vary in time (e.g. days, months, seasons, years) and space (depth, location, distance from shore) and thus sampling design should take into account temporal and spatial variability (see Kingsford 1999). Factors such as spawning, recruitment and migration routes will cause variation in number of fish within a year and between years (Kingsford 1999). Fish can undergo vertical migrations within the day and horizontal migrations over long distances (e.g. for food and reproduction) which can result in temporal and spatial variation in abundances (Kingsford 1999).

To take account of natural variability replication should always be included in a sampling design to ensure any differences between experimental treatments are because of the treatment rather than natural variation (Underwood 1997; Kingsford 1999). In studies that are deficient in replication of control/impact sites the power to generalise the results are weaker (Kingsford 1999). About three decades ago, Hurlbert (1984) reviewed various ecological experiments and noted inadequate or no replication in a number of the studies. Hurlbert (1984) defined pseudoreplication as the "... use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples maybe) or replicates are not statistically independent". Pseudoreplication can be avoided by clearly stating what the hypothesis is and planning an appropriate sampling design that would include controls, randomization and replication (Hulbert 1984; Underwood 1997).

In Chapter 3, I describe in detail the locations and sampling design for this thesis. I used static underwater video camera to observe fish around sea cages and hook and line to extract the fish of interest. During fieldwork conducted in 2013 and 2014, fish were extracted near and away from sea cages to investigate whether there were any differences in diet, condition, lipid and FA patterns in tissue between locations. The next few subsections describe in more details an overview of methodologies related to diet determination, condition and lipid and FA analysis in fish.

2.1.2 Use of stomach content analysis

Stomach content analysis is a common procedure used in fish ecology to study feeding behaviour of fishes (Hyslop 1980). Although stomach content analysis is a simple and quick method there are some drawbacks. For example, eggs and larvae are digested and evacuated faster and thus not easily identified, hard parts of prey (e.g. shells of crustaceans, heads of fish etc.) may not be consumed or can be eroded during digestion,

other items may not be identified accurately because of digestion (Hyslop 1980; Iverson et al. 2004; Kelly and Scheibling 2012). Moreover, stomach content analysis gives a snapshot of the most recent items consumed by the animal and therefore a number of other techniques (e.g. fatty acid (FA) analysis) have been developed to study the diet of animals over longer periods of time (Hyslop 1980; Dalsgaard et al. 2003; Iverson et al. 2004).

2.1.2.1 Occurrence of waste feed in wild fish

Stomach content analysis has been used in a number of studies to evaluate fish farming impacts on the diet of wild fish. A number of wild fish have been reported to consume waste feed from fish farms (Uglem et al. 2014). Fernandez-Jover et al. (2008) reported that majority of the dominant farm-aggregating species near fish farms in the Meditteranean Sea consume waste feed.

A few studies have attempted at quantifying the waste feed in the gut of the wild fish near fish farms. For example, Skog et al. (2003) reported that the gut contents of saithe caught next to cages in Norway consisted mainly of 46% waste feed. Dempster et al. (2011) quantified the stomach content of saithe and cod caught near salmon farms in Norway and found that waste feed accounted for 71% and 25% of the diet by mass of saithe and cod, respectively. Fernandez-Jover et al. (2007a) reported food pellets (> 90% of wet mass) in the stomach of horse mackerel (*Trachurus mediterraneaus*) associated with fish farms in the Mediterranean Sea.

2.1.3 Biological condition

Body condition is a term used to describe the overall health of an animal (Stevenson and Woods 2006). Condition indices are also used to represent the stored energy (e.g. lipid) in an animal (Hayes and Shonkwiler 2001). Various biochemical, bioenergetics and morphometric indices are used to indicate the condition of an organism (Stevenson and Woods 2006). Biochemical indices (e.g. proximate body constituent analysis) can be used to measure lipid or protein content; however these indices require lots of time and expenses and are destructive (Crossin and Hinch 2005; Stevenson and Woods 2006). Bioenergetic methods measure the relative amount of lipid in an organ of an animal. For example, hepatosomatic index (HSI) which is a measure of the liver mass relative to body mass of the fish is often used as an indicator of stored energy in fishes that store energy

in their liver (e.g. gadoid fishes) (e.g. Lambert and Dutil 1997). In other fish species (e.g. salmon and clupeids) that store energy around the inner organs (e.g. intestines) and muscle, the fat can be measured in these organs using electronic instruments such as fatmeter (Kent et al. 1992). Bioenergetic methods are relatively simple to perform and can be destructive (e.g. HSI) or non-destructive (e.g. fatmeter). However, such methods do not account for the total energy storage when the individuals store energy in more than one organ (McPherson et al. 2011).

Commonly used condition indicators are morphometric indices which are favoured because they are cheap to perform, simple, and non-lethal (Stevenson and Woods 2006). Fulton's condition index (FCI), body mass divided by the cube of the body length, is widely used index based on the assumption that heavier fish for a given length are in better condition (Fulton 1904; Froese 2006). The FCI is based on the assumption that fish grow isometrically (length is raised to the 3rd power) which applies to a number of fishes but not all (Froese 2006; Stevenson and Wood 2006). Fish grow isometrically when they retain their body proportionality as juveniles and adults and allometrically when some body parts change with respect to the whole body. Other morphometric measures include length mass relationships such as:

$$W = aL^b \qquad (eq.\ 2.1)$$

where W = mass (g), L = length (cm), a and b are coefficients which are useful in determining whether species exhibit isometric or allometric growth (Froese 2006). The parameter a is related to the body shape (e.g. fusiform, eel-like, elongated, short-deep) whereas the parameter b indicates allomteric/isometric growth. If b = 3 growth is isometric, if b > 3 or b < 3 growth is allometric (Froese 2006).

Stevenson and Woods (2006) argue that morphometric indices such as FCI compare the health of one population to another but there is a lack of an established definition for "healthy". Nervertheless, FCI and HSI are reliable indicators in detecting differences between fish caught near and away from cages (e.g. Fernandez-Jover et al. 2007a; Dempster et al. 2011). In this thesis, I used a combination of indices (FCI, HSI, lipid content) to detect aquaculture effects on wild marine fish.

2.1.4 Lipids and fatty acids in wild fish

In this subsection, I give an overview of FA nomenclature and the use of FAs as biomarkers in detecting aquaculture impacts on wild fish populations.

2.1.3.1 Overview of fatty acids in fish

Lipids are heterogeneous group of compounds that are extractable in nonpolar organic solvents (e.g. chloroform, benzene, ether etc.) and are relatively insoluble in water. Fatty acids (FAs) are a group of lipids that have the general formula $CH_3(CxHy)COOH$; a terminal methyl group (CH_3), a carbon chain, and a terminal carboxyl group (COOH) (Jobling 2001). FAs can be saturated (SFA), hydrocarbon chain has no double bonds, or unsaturated where the hydrocarbon chain has more than one double bond (e.g. monounsaturated (MUFAs) = 1 double bond, polyunsaturated (PUFAs) = 2-6 double bonds) (Jobling 2001; Budge et al. 2006). FAs are named based on chain length (number of carbon atoms), degree of unsaturation (number of double bonds) and position of the double bond. There are two (n- or Δ) nomenclature systems used for FAs.

In the n-nomenclature, the position of the first double bond is given by (n-x) notation and counting starts from the methyl end. For example, 22:5(n-3) is a FA with 22 carbon atoms and 5 double bonds starting after the third carbon from the methyl end. In most PUFAs the double bonds are separated by a single methylene group (CH_2). In the alternative nomenclature the double bonds are counted starting from the carboxyl end (Bergé and Barnathan 2005). I used the n-nomenclature in this thesis as it is the most commonly used nomenclature in aquaculture. Trivial and common names of main fatty acids used in Chapters 4 and 5 are found in Table 2.1.

Table 2.1 Trivial name, n-designation and systematic name of fatty acids used in this thesis (source Chow 2008).

Trivial name	n-designation	Systematic name
SFAs		
14:0	Myristic acid	Tetradecanoic acid
16:0	Palmitic acid	Hexadecanoic acid
18:0	Stearic acid	Octadecanoic acid
MUFAs		
16:1n-7	Palmitoleic acid	9-hexadecenoic acid
18:1n-7	Vaccenic acid	11-Octadecenoic acid
18:1n-9	Oleic acid	9-Octadecanoic acid
20:1n-9	Gadoleic acid	11-Eicosenoic acid
22:1n-11	Cetoleic acid	11-Docosenoic acid
n-3 PUFAs		
18:3n-3	Linolenic acid	9,12,15-Octadecatrienoic acid
18:4n-3	Stearidonic or moroctic acid	6,9,12,15-Octadecatetraenoic acid
20:5n-3	Timnodonic acid	5,8,11,14,17-eicosapentaenoic acid
22:5n-3	Clupanodonic acid	7,10,13,16,19-docosapentaenoic acid
22:6n-3	Cervonic acid	4,7,10,13,16,19-docosahexaenoic acid
n-6 PUFAs		
18:2n-6	Linoleic acid	9, 12-Octadecadienoic acid
20:4n-6	Arachidonic acid	5,8,11,14-eicosatetraenoic acid

Lipids and their constituent FAs play important roles in a number of physiological processes including source for metabolic energy for growth, reproduction, embryonic and yolk-sac larval development, membrane structure and functions, production of small hormone-like compounds or eicosanoids, and transcriptional control of lipid homeostasis (reviewed by Tocher 2003). FAs such as SFAs and MUFAs are the main substrates for energy whereas PUFAs are structural components for cell membranes, and other functions such as eicosanoid production which are involved in a number of physiological process such as blood clotting, immune and inflammatory response, renal and neural functions, reproduction, and cardiovascular tone (see Tocher 2003).

Digestion of lipids in fish starts mainly in the proximal part of the intestines. The main product of the lipid digestion are free fatty acids. Lipids are then transported from the intestines to the liver (reviewed by Tocher 2003). Biosynthesis of lipids starts with mitochondrial two-carbon organic compound acetyl-CoA as a carbon source (see Tocher 2003). In the cytoplasm, the pathway is catalysed by FA synthetase multienzyme complex. The two endogeneously synthetised FAs, 16:0 and 18:0, undergo elongation and/or desaturation reactions to obtain longer and/or unsaturated FAs, respectively (see

Tocher 2003) (Figure 2.1). Desaturases are enzymes that facilitate the introduction of double bonds. However, these enzymes cannot introduce double bonds before C9 and thus 18:2n-6 (linoleic acid) and 18:3n-3 (α-linolenic acid) cannot be synthetized. These two FAs need to be obtained from the diet. Once obtained from the diet 18:2n-6 and 18:3n-3 can be further elongated and desaturated to produce PUFAs, such as 20:4n-6 (arachidonic acid), 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid) (Figure 2.1). PUFAs, mainly 22:6n-3 and 20:5n-3 are essential for most marine fishes as they are unable to produce them in sufficient quantities (see Tocher 2003). The conversion of 18:3n-3 to 22:6n-3 and 20:5n-3 by Δ5 and Δ6 desaturase and FA elongases in marine fish is poor (Bell and Tocher 2009). 20:5n-3 and 22:6n-3 are obtained from microalgae at the bottom of the food chain (Bell and Tocher 2009). In farmed marine carnivorous fish these essential FAs need to be supplied in the diet.

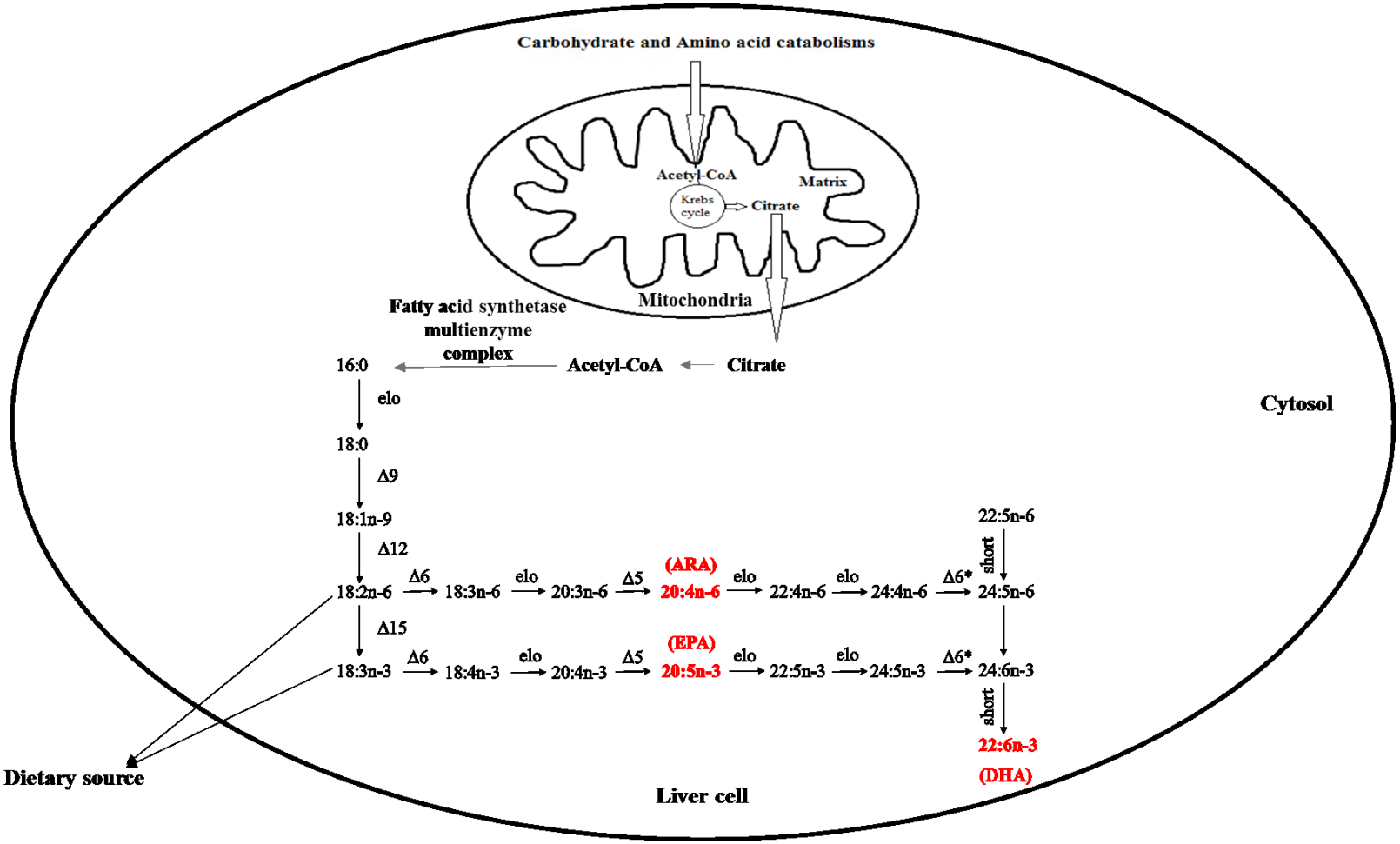

Figure 2.1 Biosynthesis of n-3 and n-6 polyunsaturated fatty acids from C18 precursor in the liver cells. Fatty acyl desaturases: Δ5, Δ6, Δ6*, Δ9, Δ12, Δ15. Fatty acyl elongases: elo. Short: chain shortening, ARA: arachidonic acid, EPA: eicosapentaenoic acid and DHA: docosahexanoic acid. Modified from Bell and Tocher (2009).

2.1.3.2 Use of fatty acid as biomarkers

In marine environments, lipid energy is transferred from low trophic levels such as microalgae to the next trophic level which is zooplankton and then to higher trophic levels such as fish (see Parrish 2013). Lipids are extensively used as biomarkers in food web ecology (see reviews by Dalsgaard et al. 2003; Bergé and Barnathan 2005; Kelly and Scheibling 2012; Parrish 2013). The main reasoning behind the use of FAs as biomarkers is that groups of primary producers possess unique FAs or ratios of FAs and that this can be conservatively transferred through the aquatic food web (see reviews by Dalsgaard et al. 2003; Bergé and Barnathan 2005; Kelly and Scheibling 2012; Parrish 2013). A number of reviews on the marine FAs occurrence, their roles and analytical methods are available (e.g. Ackman 1989; Christie 2003; Dalsgaard et al. 2003; Bergé and Barnathan 2005). FAs have been used as dietary biomarkers in pelagic, microalgal-based food webs (see review by Dalsgaard et al. 2003) and fewer studies have been conducted for benthic food webs (reviewed by Kelly and Scheibling 2012). FA analysis has been applied in evaluating the impact of aquaculture ingredients in the feed on the various aquatic organisms (e.g. shrimps (Olsen et al. 2009), sea urchins (Cook et al. 2000), mussels (Gao et al. 2006)).

2.1.3.3 Use of fatty acids biomarkers and wild fish aggregations

A number of studies have used terrestrial FA biomarkers to assess whether coastal fish farming influences wild marine fish in the vicinities of the sea cages (reviewed by Fernandez-Jover et al. 2011b; see also Arechavala-Lopez et al. 2011, 2015a; Izquierdo-Gómez et al. 2015).

Marine carnivorous farmed fish such as (e.g. Atlantic salmon (*Salmo salar*), gilthead seabream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*)) require a sufficient dietary supply of FAs such as 22:6n-3, 20:5n-3 and 20:4n-6 for optimal growth and health status. The aquaculture industry has supplied these dietary needs by using fish oil. As the worldwide capture fisheries have stagnated there is uncertainty in the production of fish oil and therefore the aquaculture industry is exploring alternative sources such as vegetable oils (e.g. soybean, rapeseed, linseed, palm oils) (Tacon and Metian 2008). However, vegetable oils are rich in 18:2n-6 and 18:3n-3 but lack n-3 PUFAs (20:5n-3, 22:6n-3) (Turchini et al. 2009). Similar to cultured fish, wild fish incorporate these FAs into their tissues as a result from feeding on waste feed from fish

farms. Therefore, influence of fish farming on wild fish populations can be detected using FAs such as 18:2n-6 and low ratio of n-3/n-6 as biomarkers (reviewed by Fernandez-Jover et al. 2011b).

Skog et al. (2003) reported that saithe captured near salmon farms in Norway had a muscle FA profile similar to that of the feed used for farmed fish. The muscle tissues of saithe had elevated levels of 18:2n-6, 18:1n-9, 18:3n-3 and low n-3/n-6 PUFA ratio. Similar results were obtained by Arechavala-Lopez et al. (2015a) in both the liver and muscle of saithe caught near fish farms in Norway. In another study in Norway, Fernandez-Jover et al. (2011a) found elevated levels of terrestrially derived FAs (18:2n-6, 18:1n-9) and reduced levels of 22:6n-3 in the tissues (muscle and/or liver) of gadoid species (saithe and cod) caught near salmon farms indicating fish farming influence.

Izquierdo-Gómez et al. (2015) reported that fish farms in the Mediterranean Sea attracted various pelagic and benthic fish and the consumption of waste feed or prey resulted in modified levels of FAs. The authors, using the FA 18:2n-6 as a biomarker, found that some of the wild fish that have visited the fish farms were captured by local artisanal fisheries. Commercially important horse mackerel (*Trachurus mediterraneus*) and bogue (*Boops boops*) were both found to consume waste feed near fish farms in the Mediterranean Sea which resulted in elevated levels of FAs such as 18:2n-6 and 18:1n-9 and lower levels of 22:6n-3 as compared to their wild counterparts (Fernandez-Jover et al. 2007a; Arechavala-Lopez et al. 2011). Similarly, Ramírez et al. (2013) found increased levels of 18:2n-6, 18:1n-9 and 18:3n-3 and lower levels of 20:4n-6 and 22:6n-3 in bogue influenced by aquaculture in the Canary Islands. However, the authors noted that bogue sampled near sewage waters had also elevated levels of 18:2n-6 and 18:1n-9 and thus 18:3n-3 was suggested to be a better FA biomarker to indicate influence of fish farms.

Fernandez-Jover et al. (2009) reported that zooplankton were the predominant prey for juvenile mugilid (*Liza aurata*) and juvenile sparid (*Oblada melanura*) associated with sea cages in the Mediterranean Sea and FA changes in zooplankton were also reflected in the FA profiles of the juvenile fish.

As Fernandez-Jover et al. (2011b) pointed out no single FA can be used as the sole indicator for fish farming influence on wild fish because some of these terrestrial biomarkers (e.g. 18:2n-6) are also found at low levels in marine food webs. Additionally, some of the FAs used as biomarkers could originate from sewage or agriculture (e.g. Ramírez et al. 2013). A number of various multivariate approaches (e.g. principal

component analysis (PCA) (Skog et al. 2003; Fernandez-Jover et al. 2011a), linear discriminant analysis (LDA) (Fernandez-Jover et al. 2011a; Olsen et al. 2015)) have been applied to discriminate the origin of fish or the impact of plant-derived FAs on wild fish (reviewed by Fernandez-Jover et al. 2011b).

There is lack of knowledge on factors such as the minimum time wild fish spend around the fish farms, the amount of waste feed consumed, biology and metabolism of lipids for each species that would induce significant changes in FA profiles (Fernandez-Jover et al. 2011b). Migratory horse mackerel captured around fish farms in the Mediterranean Sea had modified FAs within 3-4 months (Fernandez-Jover et al. 2007a). In a laboratory experiment, Olsen et al. (2015) investigated the influence of fish farm waste on wild fish by using a diet-switch study where cod fry were fed either salmon, cod (control diet) or herring diet for 121 days. Salmon and cod diet had similar fatty acid profiles but the salmon diet contained higher vegetable oils. The herring diet, representing the natural diet of cod, was higher in marine oils. The authors reported that cod fed the salmon diet had elevated levels of 18:2n-6 and 18:3n-3 whereas the cod fed the herring diet had elevated levels of 20:1n-9 and 22:1n-11. Terrestrially derived FAs (18:2n-6 and 18:3n-3) are more slowly incorporated (day 69) in the muscle of cod than marine FAs (20:1n-9, 22:1n-11) (day 26 of 121 days). Regost et al. (2003) reported that when turbot (*Psetta maxima*) was fed vegetable oil based diet for 13 weeks and then was switched to fish oil based diet the FA profiles after two months did not fully recover a similar FA profile as the initial state of the fish. The authors suggested that the time required to adapt to a new diet is longer for this species.

2.2 Modelling approaches

The majority of the literature related to impacts of aquaculture activities on wild fish communities focuses on direct impacts related to the consumption of waste feed using empirical approaches. There is a lack of studies on extrapolating the fish farming impacts at the population level and only few studies evaluate impacts at the ecosystem level with emphasis on wild fish around fish farms (e.g. Díaz López et al. 2008; Bayle-Sempere et al. 2013). As Uglem et al. (2014) noted in their review more studies are needed to assess the ecological processes at single-species levels and across trophic levels in order to understand the overall impact of sea cage fish farming.

In this section, I focus mainly on the role of modelling as a tool to study impacts of aquaculture activities on wild fish associated with sea cages at the population and ecosystem levels. I give a brief overview of statistical modelling used in Chapters 4-6.

The two main models used in this thesis are single-species population models (Chapter 7) and ecosystem based models (Chapter 8). I give only an overview of the models and more details are given in the corresponding chapters.

2.2.1 Role of modelling

A model is a simplification of a real world process and can be used as a tool to answer various research questions (Jørgensen and Bendoricchio 2001). Empirical methods involve collection of data in the field or laboratory followed by analysis of the data using various statistical models without much consideration of the underlying theory whereas theoretical methods use a number of unrealistic assumptions needed to build mathematical models that can provide understanding of ecological patterns (Codling and Dumbrell 2012). Both methods are needed to answer the addressed research questions as using only empirical method without theory is pointless and building theoretical models without evaluation against real data is also meaningless (Codling and Dumbrell 2012). A major advantage of using theoretical models is the possibility to design numerous scenarios which is often a limitation in field or laboratory studies because of logistics (Codling and Dumbrell 2012).

2.2.1.1 Statistical modelling

Many of the studies related to fish farming impacts on wild fish communities use hypothesis driven modelling approach (e.g. Skog et al. 2003; Fernandez-Jover et al. 2007a; Dempster et la. 2011). In brief, once a research question is conceived it is framed in terms of two hypothesis. For example, if two populations are to be compared the null hypothesis assumes no difference between the population means (H_o: $\mu_1 = \mu_2$) whereas the alternative hypothesis assumes that there is a difference between population means (H_a: $\mu_1 \neq \mu_2$). Once the data is collected, a test statistic, a random variable, is calculated and compared to a hypothesised null distribution to check whether there is evidence to reject or accept the null hypothesis. Based on the test statistic values, the data are either consistent or not consistent with the stated null hypothesis. If the test statistic is often obtained by chance then there is no reason to reject the null hypothesis whereas if the test

statistic is rarely encountered by chance then the null hypothesis is rejected. An arbitrary value (α), probability of 0.05 (1 in 20) is used as a cutoff for statistical significance or not (Underwood 1997).

To test the hypothesis different models can be applied to the collected data. A common model is the linear regression model which is defined by:

$$Y_i = \alpha + \beta \times X_i + \varepsilon_i \text{ where } \varepsilon_i \sim N(0, \sigma^2) \quad (eq.\ 2.2)$$

Y_i is the dependent variable, X_i is the independent variable, α is the population intercept, β is the regression coefficient or the population slope. The residuals ε_i are part of the total variation that are unexplained by the regression model. The residuals are assumed to be normally distributed with mean 0 and variance σ^2 (Zuur et al. 2009). Using a sample of data to make inferences about the population is based on assumptions. Assumptions for a linear regression model include normality of the residuals, homogeneity of residuals, and independence (Zuur et al. 2009).

Often, however, model assumptions fail with ecological data which may or may not have significant impact on the conclusions (Zuur et al. 2010). Zuur et al. (2010) suggested that to avoid problems related to failure in model assumptions is the exploration of data using different graphical tools (e.g. boxplots, scatter plots). The presence of outliers, values that are too large or small with respect to the rest of the data could affect the model assumptions by declaring significant differences when there are none (Zuur et al. 2010). Outliers could be removed to improve the model and the consequent ecological conclusions (Zuur et al. 2010).

Homogeneity of variance is an important assumption for the linear regression models (Zuur er al. 2010). Plotting the residuals vs the fitted values of the linear regression models should show similar residual variances (Zuur et al. 2010). Depending on the data transforming the response variable may remedy the lack of homogeneity of variances (Zuur et al. 2010). If transformations (e.g. logarithmic, square-root) are not appropriate then choosing models (e.g. generalised least squares) that do not require homogeneity of variances may be more appropriate (Zuur et al. 2010). Transformations of the original data are not always advisable as it may lead to differences in conclusions between transformed and non-transformed data (see Zuur et al. 2010 and references therein).

In linear regression models normality of the residuals is also one of the assumptions (Zuur et al. 2010). Gelman and Hill (2007) noted that the normality of the errors in regression models is one of the least important assumptions. Nevertheless, if normality is to be assessed quantile-quantile (Q-Q) plot is a useful graphical technique (see Chapters 4-6). If the normality assumption is violated transforming the data may be an option or using more advanced models (e.g. generalised least square) that do not require this assumption (Zuur et al. 2010).

The use of non-parametric techniques is also another option when the assumptions (e.g. normality) of parametric techniques are violated (see Chapter 4) (Sheskin 2004). In the parametric techniques the researcher tests for the differences between means of groups whereas in the nonparametric techniques the location statistic is the median (central value in a distribution where above and below lie an equal number of values). In nonparametric testing there is an overall lack of precision in how two groups differ and therefore should be used as last resort.

Mixed effects models are a powerful statistical tool that are often used to analyse data structured into groups (e.g. nested data) (Zuur et al. 2009). Mixed effects modelling is of particular importance to aquaculture. It can be used to model the random variation between farms. Farms are subject to variation that is essentially random (in that the independent variables do not describe it), such as variation in husbandry and management practices). Taking the mixed effects approach allows us to model how farms vary from other control/reference sites in general, despite each having unique features; whereas the fixed effect approach deals with each farm as having its own specific features and the notion of a typical farm is absent from the model. Mixed effects models can also be used to model the random variation between tanks in controlled experiments that use replicate tanks.

Mixed effects model is defined as:

$$Y_i = X_i \times \beta + Z_i \times b_i + \varepsilon_i \qquad (eq.\ 2.3)$$

Y_i is the dependent variable, both terms the fixed $X_i \times \beta$ and the random $Z_i \times b_i$ are part of the explanatory variables and ε_i is the residuals (Zuur et al. 2009). The random part of the model allows the incorporation of a nested structure in the data. The random effects are assumed to be normally distributed with a variance [$b_i \sim N(0, D)$], the residuals are

also assumed to be normally distributed [$\varepsilon_i \sim N(0, \sigma^2)$] with covariance matrix $\Sigma_i, b_1, \ldots b_N$, $\varepsilon_{1, \ldots\ldots, } \varepsilon_N$ are also independent (Zuur et al. 2009).

If the assumptions of the linear mixed effect models are violated and if excluding outliers and/or transformation of the response variable do not improve the models then more advanced models may be appropriate. For example, models such as generalised linear mixed effects models (GLMMs) (e.g. Bolker et al. 2008) that are more flexible with nonnormal data and include nesting structure. However, the choice of model depends on the data. In Chapter 5 and 6, I used linear mixed effect models despite some moderate violations in the model assumptions because conceptually these models capture the pattern of variation that the body of theory suggests the data should follow (see the corresponding Chapters for further discussion).

Multivariate modelling approach is also another powerful tool to simultaneously analyse patterns in data that involve a number of variables. There are many different multivariate modelling approaches (e.g. Greenacre and Primicerio 2013). These techniques have proved to be valuable in aquaculture where the multiple variables are measures of different fatty acids or other chemicals; as well as in ecology where the variables are abundances of different species. In this thesis, I mainly use principal component analysis (PCA) (Chapter 4), linear discriminant analysis (LDA) (Chapter 5) and multiple correspondence analysis (MCA) (Chapter 6).

2.2.1.2 Single species population models

Matrix population models are important tools used in studying the demography of age-, stage- or size- structured populations, wildlife management and conservation of endangered species (see Crouse et al. 1987; Caswell 2001; Fieberg and Ellner 2001; Andersen et al. 2004; Rogers-Bennett and Leaf 2006). One of the most popular matrix models in population ecology is the Leslie population matrix model (Leslie 1945; Caswell 2001). Leslie population matrix model is an age-structured model that incorporates fecundity and survival rates of female individual classes within a population (Leslie 1945; Caswell 2001). The output of the Leslie matrix gives a range of parameters that are useful in understanding population dynamics of the species and also to compare different populations and species. For example, one main output of the matrix is the population growth rate (λ) and if $\lambda = 1$ the population is stable, if $\lambda > 1$ the population is increasing over time and if $\lambda < 1$ population is declining (Caswell 2001).

Sensitivity and elasticity analysis are tools commonly applied in matrix population models that allow comparison of contributions of vital demographic rates (e.g. survival and fecundity) to population growth rate (Benton and Grant 1999; Caswell 2001). The sensitivity analysis is the absolute change in population growth rate as a result from absolute changes in vital rates (e.g. survival and fecundity) whereas elasticity analysis (or proportional sensitivity) is the proportional change in population growth rate as a result of proportional change in vital rates (de Kroon et al. 2000). For instance, if the survival of juveniles has a high elasticity then a small proportional decrease in survival will lead to large proportional effects on the population growth rate. On the other hand, if the survival of juveniles has a low elasticity then large changes in survival will have a relatively small effect on the population growth rate (Benton and Grant 1999; Caswell 2000). Sensitivity and elasticity analysis can be used in supporting decisions regarding the management and conservation of species (Benton and Grant 1999; Caswell 2000). Elasticity analysis is advantageous to use when little data are available to model a species. The elasticity analysis can provide information on the data needed to be collected in order to improve management of the species (Heppell et al. 2000).

Based on observations and data collected during fieldwork conducted in 2013 and 2014 (see Chapter 3) single species models were developed for mackerel and whiting sampled near sea cages (see Chapter 7).

Single-species models such as Leslie population model are a simplification of a rather complex reality. In a single species model the species modelled is in reality one of many members of a large interacting complex ecosystem which is composed of many different species and nutrients. Thus, in order to capture a more realistic view of the ecosystem there has been an increasing interest in the use of ecosystem models (Fulton et al. 2003; Latour et al. 2003; Pikitch et al. 2004).

2.2.1.3 Ecosystem based models

A popular ecosystem modelling approach is Ecopath with Ecosim (EwE)[2] with the first Ecopath model built in the 1980s by Polovina (1984) (Christensen and Walters 2004; Christensen et al. 2005; Heymans et al. 2016). Ecopath is based on the principle that for each functional group (species or groups of species) ranging from low to high trophic levels the energy removed from a group by predation or fishing needs to be balanced by

[2] http://ecopath.org/ [Accessed: 2 February 2018].

the energy consumed by the group (Coll et al. 2009). The initial Ecopath model was modified and extended to include modules such as Ecosim (time-dynamic simulation) and Ecospace (spatial-temporal dynamics) (Christensen and Walters 2004; Colléter et al. 2015; Heymans et al. 2016). The EwE modelling approach is a popular tool which is also reflected in the number of increasing published models (> 400; Ecobase[3] online repository for Ecopath models). In addition to providing simplified description of complex systems, the model building is relatively easy to use (provided data is available) which has attracted many researchers to use the tool (see Colléter et al. 2015; Heymans et al. 2016). Although majority of EwE models have focused on fisheries related topics in the Northern and Central Atlantic Ocean the use of the models has expanded to other regions (e.g. Indian and Antarctic Oceans) and research topics (e.g. pollution, marine protected areas) (see Colléter et al. 2015). The EwE modelling approach has also been used in evaluating the impacts of aquaculture activities such as fish farming (Díaz López et al. 2008; Forrestal et al. 2012; Bayle-Sempere et al. 2013), and shellfish farming (Jiang and Gibbs 2005; Leloup et al. 2008) on the food web.

An ecosystem-based model was developed to detect aquaculture effects on wild fish communities around fish farms in a sea loch (see Chapter 8).

2.3 Conclusions

A critical issue in evaluating the impacts of aquaculture is in establishing an evidence base (e.g. population surveys and biological condition indices) to assess the balance between positive and negative effects on a population, and then using modelling techniques to weigh these positive and negative effects against each other. As no empirical or modelling approach is ideal it is necessary to have a combination of approaches (either empirical, modelling or both) to inform our understanding of the effects of aquaculture on wild marine fish populations.

[3] http://sirs.agrocampus-ouest.fr/EcoBase/ [Accessed: 2 February 2018].

CHAPTER 3
FIELD AND LABORATORY STUDY METHODS

3.1 Overview

Both empirical and modelling approaches were used to evaluate the aquaculture impacts on sampled wild fishes at the individual, population, and ecosystem levels. The selected sites in this research were based on the resources available at the start of the research project. Some restrictions were related to the distance between the initially chosen sites and the University of Stirling. Other issues related to logistics led to abandoning some of the initial objectives of the research proposal. For example, the number of sites to be studied were three similar fish farms or salmon farms with corresponding reference sites across the West coast. Additional data that were to be collected for Chapters 4-6 included fish abundances near and away from farms, fish sex, otoliths, gonad mass, heavy metals/contaminants, and/or parasites. Some trial plankton and seaweed sampling was conducted mainly for Chapter 8; however the overall output of the trials was not found useful. The overall objectives of the research were achieved despite some of the encountered limitations.

In 2013, a study was conducted near a fish farm (halibut farm) in Loch Melfort. The purpose of the study was to test the underwater video equipment and collect fish near and away from the sea cages. Data collected was used to evaluate whether there were any differences in diets, biological condition, total lipid and fatty acids in muscle and liver tissues of mackerel and saithe caught near and away from the sea cages. Result of this work are presented in Chapter 4. The study was extended in 2014 to include a second farm (salmon farm), additional reference sites for each species and increased number of sampled fish (mackerel and whiting) (Chapter 5). In Chapter 6, all the data collected (diets, condition indices) for both years (2013 and 2014) were combined for mackerel, whiting and saithe to give insights into whether coastal sea cages act as ecological traps or production sites. Empirical approaches used to collect data were necessary to inform the modelling work to enable robust scaling up from individual level changes to population (Chapter 7) and ecosystem level effects (Chapter 8). As there is limited knowledge of ecological interactions between coastal aquaculture activities and wild fish populations in Scotland it was necessary to collect the data described in this chapter.

This chapter describes the sampling sites (section 3.2) and methodologies (sections 3.3-3.6) used to collect data necessary for Chapters 4-8. Underwater video recordings were used to observe wild fish around two fish farms (section 3.3). Macrobenthic sampling was conducted (section 3.4) near one fish farm for descriptive purposes for the ecosystem-based model built in Chapter 8. During sampling events in 2013 and 2014 environmental data was collected (section 3.5). Methods of fish extraction and processing can be found in section 3.6. Appendix A contains additional information on the number of fish caught near and away from the two fish farms.

3.2 Farm sites and farm characteristics

Two fish farms located in two lochs (Loch Melfort (Figures 3.1 and 3.2) and Loch Leven (Figures 3.3 and 3.4)) were selected based on the cooperation of fish farmers and the accessibility to the selected sites.

All maps for the selected sites used in Chapters 3-6 were generated using the open-source software R (R Core Development Team (2016)) and libraries rgdal (Bivand et al. 2016), ggplot2 (Wickham 2009), rgeos (Bivand and Rundel 2016), and maptools (Bivand and Lewin-Koh 2016) and Global Administrative Areas (GADM) database[4].

3.2.1 Loch Melfort

Loch Melfort (Figure 3.1) is a fjordic type small sea-loch that extends about six km in length and has a maximum depth of 73 metres. The sea loch has a single sill of 2.1 km in length and an average depth of 19 metres and is sheltered from the open ocean by the islands of Luing and Shuna (Edwards and Sharples 1986). The catchment area is 73 km^2. The fresh/tidal flow per thousand is 10.2. The flushing time for Loch Melfort is nine days. Tidal range is 2.3 metres (see Edwards and Sharples 1986). Loch Melfort has several aquaculture facilities rearing fish (sea grown rainbow trout, Atlantic halibut, and common mussels (*Mytilus edulis*)). Other shellfish cultured on rafts include pacific oysters (*Crassostrea gigas*), native oysters (*Ostrea edulis*), king scallops (*Pecten maximus*), and queen scallops (*Aequipecten opercularis*); however cultivation of these four species has not reached commercially viable levels (Scottish Sanitary Survey Report 2015). The selected farm (Figure 3.2; 56.2475 N, 5.5145 W) for this thesis was located in the upper end of Loch Melfort, at Kames Bay. The farm was about two metres off the shore in water

[4] http://www.gadm.org/ [Accessed: 2 February 2018].

depth of 14-23 metres. The farm was accessed from the shore by a jetty. The farm consisted of six circular cages each having a diameter of 22.3 metres and 7-8 metres depth. The farm produces Atlantic halibut with maximum consented biomass of 250 tonnes/year. Sampling and underwater video recording took place in September 2013 and July/August 2014 at the sea cages in Kames Bay.

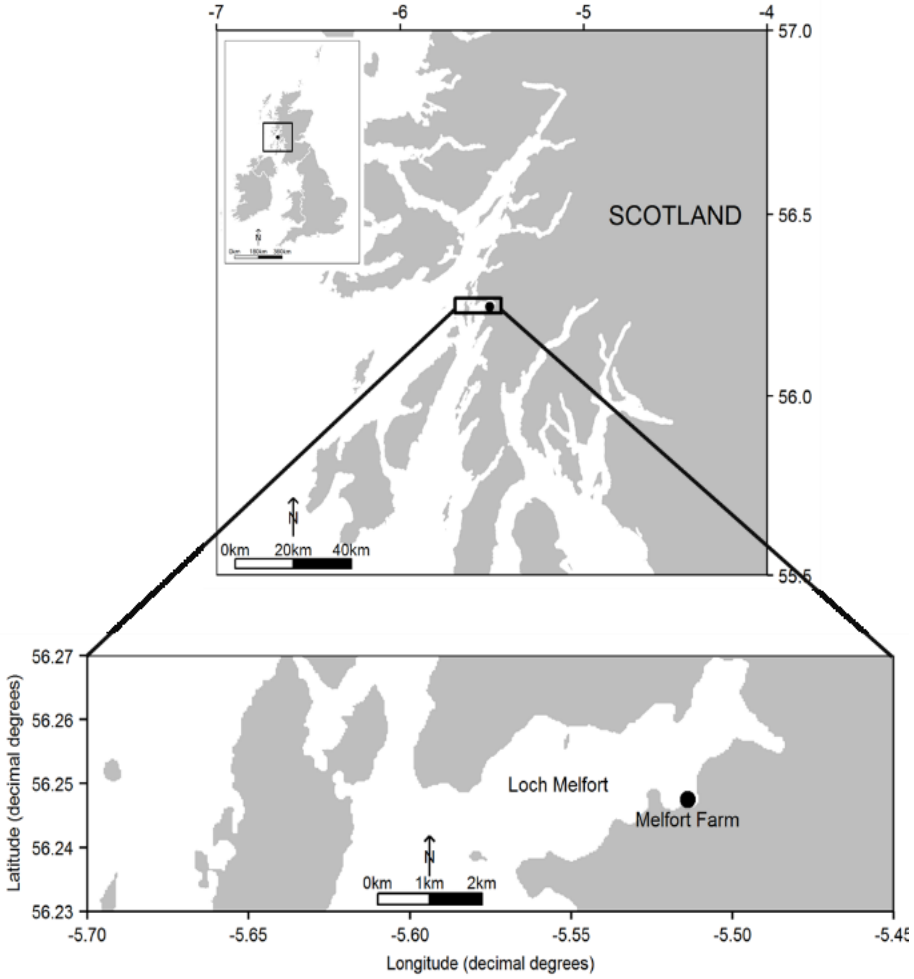

Figure 3.1 Loch Melfort, West Coast of Scotland. Farm is noted with a black dot.

Figure 3.2 Selected farm at Kames Bay, Loch Melfort.

3.2.2 Loch Leven

Loch Leven (Figure 3.3) is a sea loch of 13.4 km in length and a maximum depth of 62 metres. The catchment area is 338 km^2. The loch is sheltered from all but west winds and has five sills. The fresh/tidal flow ratio per thousand is 40.5 and the flushing time is three days. Tidal range is 3.7 metres (see Edwards and Sharples 1986). The selected farm (Figure 3.4; 56.6880 N, 5.1375 W) is about 120 metres off the shore at an average depth of 25 metres. The farm was accessed from the shore by a boat. The farm comprises of twelve 24 $metres^2$ steel pens and produces Atlantic salmon (*Salmo salar* L.) with maximum consented biomass of 1450 tonnes/year. Farming of common mussels is also present in the loch. Fish sampling at Leven Farm took place in July/August 2014.

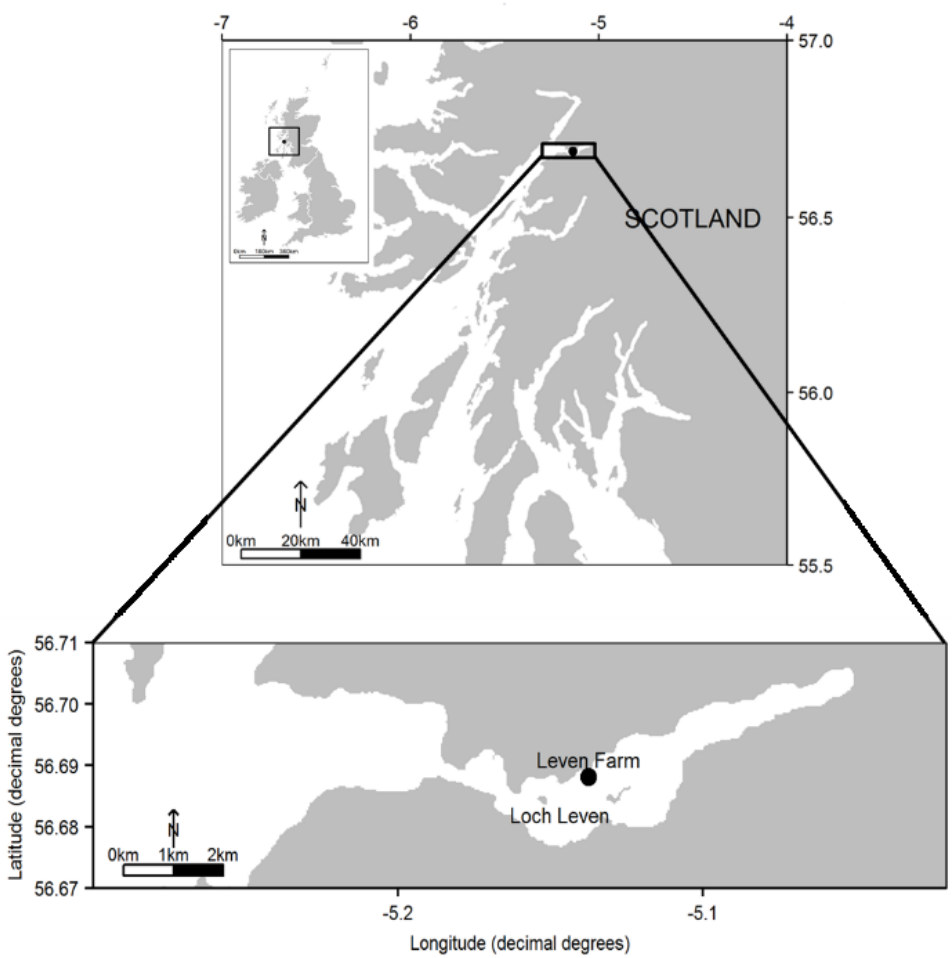

Figure 3.3 Loch Leven, West Coast of Scotland. Farm is noted with a black dot.

Figure 3.4 Selected farm at Loch Leven.

3.2.3 Comparison of selected lochs and farms

Loch Melfort and Loch Leven are both relatively small lochs. The catchment area for Loch Leven is larger than for Loch Melfort which indicates a larger freshwater input in Loch Leven. This is also indicated to some extent by the differences in salinity measurements between the sea lochs taken during the fieldwork of 2014 (see Appendix A). The flushing time (the time it takes for all or some of the water in the loch to be replaced by the tidal currents (Gillibrand 2001)) in Loch Leven is three days whereas that of Loch Melfort is nine days. The flushing time difference between the two lochs indicates that resident times for phytoplankton and nutrients is higher for Loch Melfort than for Loch Leven.

Both lochs have fish and shellfish farming. A salmon farm and a halibut farm were selected in Loch Leven and Loch Melfort, respectively. Details on farm management, locations and abbreviations used throughout the studies are given in Table 3.1. Halibut farming has a limited production as compared to salmon production (see subsection 1.8.2; Chapter 1). The maximum allowed biomass for the chosen salmon farm is almost six times more than the halibut farm production (Table 3.1). The halibut farm is located in a

very sheltered bay whereas the salmon farm is located in a well flushed area indicating that nutrients from the salmon farm will be more dispersed than those of the halibut farm. In 2013 and 2014, the halibut farm was towards the end of the production cycle (36-56 months) whereas the salmon farm was in the beginning of the production cycle (18 months) indicating differences in the diets fed to the cultured fish. At the halibut farm the feeding frequency was manual whereas at the salmon farm feeding was automated which may indicate more waste feed at halibut farm (Table 3.1). However, halibut farming often has a tarpaulin at the bottom of the cage which allows the halibut to consume settled feed and therefore less artificial feed would be lost (Gillibrand et al. 2002).

Table 3.1 Farm locations and farm management details, feed and production.

Farm management details	**Kames Bay, Loch Melfort**			**Loch Leven**	
Abbreviation	Melfort Farm			Leven Farm	
Species cultured	Atlantic halibut (*Hippoglossus hippoglossus* L.)			Atlantic salmon (*Salmo salar*)	
Dates visited	September 2013	July 2014	August 2014	July 2014	August 2014
Maximum consented biomass (tonnes/year)*	250			1450	
Actual biomass at time of sampling (tonnes)*	119	98	45	237	357
Feed offered during month of sampling (kg)*	19993	3481	3246	77121	124821
Management	Late in production cycle (1 production cycle = 36-56 months)	End of production cycle	End of production cycle	Early of production cycle (1 production cycle = 18 months)	Early of production cycle
Feeding Frequency	Hand fed three times daily			Automatic feeders	

*data obtained from: http://aquaculture.scotland.gov.uk/data/fish_farms_monthly_biomass_and_treatment_reports.aspx [Accessed: 2 February 2018].

3.3 Underwater video recordings

Underwater video recordings were initially employed in order to estimate abundances of wild fish around fish farms.

Several trials using a remotely operated vehicle (ROV) (LBV 150, SeaBotix Inc., USA) (Figure 3.5) next to cages were undertaken. However, it was found difficult to operate the ROV next to sea cages because of obstructions such as mooring ropes. Thus, trials using a standstill underwater video camera system were undertaken in 2013 and 2014.

A standstill underwater video camera system (Figure 3.6) capable of recording in the water column was used to depth of 20 metres. A video camera (Sony HDR XR160) was mounted in a housing (SEAPRO SP10, Greenway Marine, UK) capable of depths to 50 metres. The housing has external controls including zoom, on/off controls and video run and led bulb system (PP70, 12 volt, 50 watt, Led 4, wide angle of 50 degrees) fitted on flexible arms. A stainless steel frame of 44.5 cm height was engineered for attaching stainless steel poles of different lengths (1 and 2 metres) to a total depth of 20 metres, joined by screw collars. The camera system was lowered, with the assistance of two to three people into the water, by a pole to the desired depth. A rope was attached to the camera system for emergency recovery. The orientation of the camera was determined by marking the top of the pole.

Figure 3.5 Several trials were conducted to record fish around cages using a remotely operated vehicle (ROV).

Figure 3.6 Fish around cages were recorded using a standstill underwater camera system mounted in a housing operated by attaching poles and a rope to the desired depth

On September 10, 2013 various trials were conducted. The camera was attached to the jetty of the fish farm next to a cage and lowered down. Every 30 secs the camera was dropped down by 2 metres and allowed to record for a total of 2 mins while turning it 90 degrees every 30 secs. A total of three trials were conducted at three different locations and depths on the jetty using the above mentioned procedure. Two other trials were conducted by dropping the camera at a certain depth and allowed to record for 30 mins to 1 hr. One trial was conducted by dropping down the camera to just below the bottom of the sea cage of about 7.0 metres and allowed to record for 30 mins. This was done during handheld feeding of farmed halibut. Another drop down camera trial was conducted at about 1.5 metres from the shore and allowed to record for 1 hr. During the trials the tide in Loch Melfort was low. On September 11, 2013 the same procedure was repeated once at approximately the same location as in the previous day. The maximum depth reached was about 20 metres whereas the day before it was approximately 14 metres. The tide was high during recordings. The water current was strong and thus after a depth of 12 metres the poles were slightly tilting.

Following the aforementioned trials the camera system was lowered to depths of approximately 1 to 7 metres by attaching it to the jetty and allowed to record up to 4 hrs on five different occasions (September 14, 15, 16, 21, 22, 2013). Lowering the camera to depth below 7 metres was not undertaken because of poor water clarity and adverse wind and current conditions.

Using the same set up as the underwater video trials during 2013, the same procedure was repeated during 2014. Underwater videos were taken on July 19, 26 and August 23 in Loch Melfort and on July 10 in Loch Leven. It is also worth noting that one trial with baited underwater video recordings was conducted at Loch Melfort. However, as difficulties arose from logistics and bad weather the underwater video recording was stopped at both farms in Loch Leven and Loch Melfort.

Using the video camera system in the present research allowed a permanent record of the organisms around the cages. The data generated from these recordings was mainly used for qualitative analysis (see Appendix A).

3.4 Macrobenthic sampling

Macrobenthic sampling was taken mainly for qualitative analysis (see Appendix A) to be used for the ecosystem model developed in Chapter 8. Some quantitative analysis was conducted; however no statistical analysis was performed as there were no sufficient number of samples. Logistics did not allow for the extension of the study or to repeat it during the fieldwork undertaken in the summer of 2014.

3.4.1 Macrobenthic sampling

Sampling took place at seven sampling points; one beneath the cages (0 metres), and others at approximately 20, 60, 300, 500, 700 and 900 metres from the sea cages (Figure 3.7). The sampling was limited to one transect only because of the logistics (sampling time and cost). The samples along the transect were collected using a motor boat operated by farm staff. The actual points of each sample were recorded using a handheld GPS. At each sampling point three 0.045 m^2 van Veen grab samples (Figure 3.8) were taken for analysis of macrofauna and sieved through a 1 mm mesh. Samples were not obtained at distances 300 and 700 metres from the fish farms because of the rocky nature of the seabed. Samples were returned to the laboratory for identification and enumeration. Macrofauna were carefully separated from the sediment in trays under X10

magnification using an Olympus SZ51 stereo microscope (8-40X magnification). Because of logistics samples were stored in 70% v/v ethanol and analysed within 10 days of sampling. All benthic macrofauna was identified to species level when possible according to Hayward and Ryland (1990).

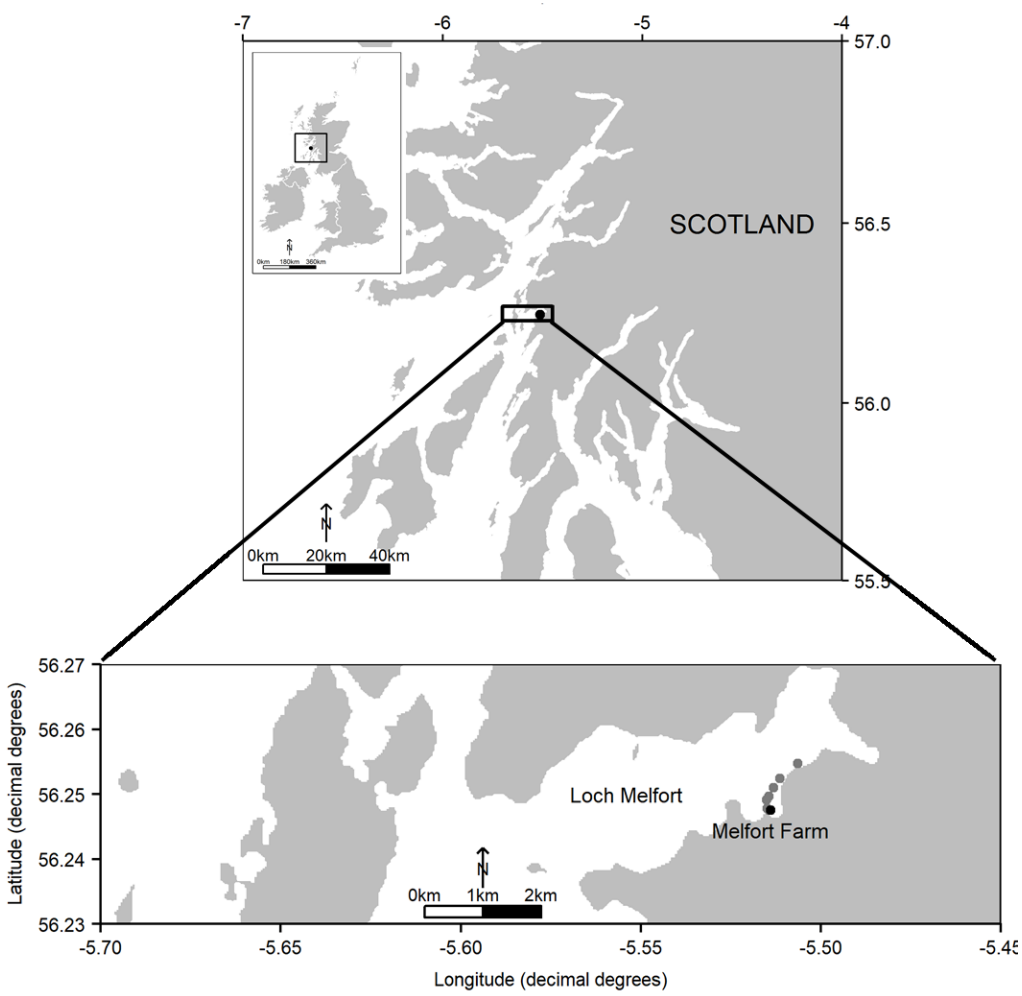

Figure 3.7 Sampling locations for benthic sampling in Loch Melfort, West Coast of Scotland. Farm is noted with a black dot. Benthic sampling locations are noted with dark grey dots.

Figure 3.8 van Veen grab samples taken at Kames Bay, Loch Melfort, 2013.

3.5 Environmental data collection

Dissolved oxygen concentration (mg/L) was measured using oxygen meter (YSI, Model 58). Temperature (°C) and salinity (ppt) were measured with a conductivity meter (WTW-Wissenschaftlich Technische Werkstätten, Model LF 58). Dissolved oxygen, temperature and salinity were measured at 2-5 metres from the surface at each sampling event next to Loch Melfort. Temperature and salinity during fieldtrips in July/August, 2014 at the farm in Loch Leven were obtained from the farm staff (see Appendix A). Logistics did not allow any of the environmental parameters to be taken on any of the reference locations during fish sampling.

3.6 Fish sampling and processing

3.6.1 Fish sampling

Fish next to Melfort and Leven farms were sampled by using baited rod and line fishing gear (Figure 3.9). Fish collection using rod and line selects for feeding fish.

Additionally, capture by towed gear beside the cages at fish farms is impractical because of possible interactions of fishing gear with the fish farm. Mackerel were caught using three hook feather rig (Shakespeare Mackerel Rig; SP 3240; "J" hooks size 1/0) placed on a monofilament main line (0.25 mm) on a conventional spinning reel and a 3 metres rod. Whiting were caught using three hook rig (Shakespeare SP 3280; "J" hooks size 2). The rig encompassed a 100 g lead at the end of the main line. The rig was placed on a monofilament main line (0.25 mm) on a conventional spinning reel and a 3 metres rod. All hooks were baited with pieces of mackerel covering the whole hook surface. Although mackerel was caught using feathers the use of bait was used to standardise the procedure as much as possible. It is worth noting that both species were caught using either the gear for mackerel or for whiting. Saithe was caught using the same gear as whiting. Fish sampling with rod and line was done between 2 and 6 hrs (8am-2pm) at each sampling occasion.

In 2013, fish sampling at Melfort farm and reference sites took place on the following days; September 14, 15, 16, 21, and 22, 2013. In 2014, fish sampling took place on July, 20, 21, 26, 27, August 15, 16, 20, 23, 24 at Loch Melfort and fish at Leven farm were sampled on July, 10, 17, 24, 31, and August, 08, 15, 21. All fish species caught during fieldwork in 2013 and 2014 can be found in Appendix A. All fish collected during fieldwork were identified using identification key (Wheeler 1978).

Figure 3.9 Fish collected using rod and line next to cages.

3.6.2 Sampling design

Based on published literature (e.g. Carss (1990)) saithe was reported as the predominant fish species near sea cages in Loch Melfort. Thus, saithe was the fish initially chosen for the studies. However, at the time of sampling and methodology used saithe was found in very low numbers compared to other fished species. Based on underwater videos and sampling methodology mackerel and whiting were the predominant fish species at both farm locations and thus were chosen for the first study described in Chapter 4. However, as logistics did not permit the sampling of whiting at a reference site in 2013 juvenile saithe was chosen instead as a preliminary juvenile gadoid model (see Chapter 4). In 2014, the study was extended to two farms each located in Loch Melfort and Loch Leven to assess the impacts of two fish farms on mackerel and juvenile whiting (Chapter 5).

Details on reference locations, location abbreviations used throughout the study, main fish species caught at each location and methods of catching are given in Table 3.2. In 2013, mackerel and saithe were sampled near a fish farm located at the upper end of Loch Melfort and a reference site for each species on the West Coast of Scotland (see Chapter 4). In 2014, three reference sites were chosen for each sampled species (mackerel and whiting) (Chapter 5). Whiting caught by fishermen at a third reference site were bigger in size compared to those caught near the two fish farms and therefore were not used in the statistical models for Chapters 5 and 6. Information on the whiting sampled from the third reference site can be found in Appendix C.

Mackerel from Isle of Luing (Reference Mackerel 1) were purchased from local fisherman at the North Cuan Seil Ferry Terminal on August 16, 2014. Mackerel from Oban bay (Reference Mackerel 2) were caught on August 10 and 23, 2014. Mackerel from Mallaig (Reference Mackerel 3) were purchased from the North West Fishermen's Association Ltd. on September 6, 2014.

Whiting from the Firth of Clyde (Reference Whiting 1) and North Minch (Reference Whiting 2) were caught on August 20, 23, 24, 2014. The whiting from reference sites were obtained from Marine Scotland and were caught using bottom-trawling. Whiting from Mallaig (Reference Whiting 3) were purchased from the same place as mackerel from Mallaig and were caught using rod and line. All data from farmed and control sites were pooled together to analyse the effect of two fish farms on sampled wild fish near the sea cages.

Table 3.2 Main fish species, mackerel, saithe, and whiting, collected and method of catching at each farm and reference locations.

Location name	Abbreviation	Sampling time	Main fish species caught (number of fish)	Method of catching fish	Distance to closest fish farm (km)
Loch Melfort	Melfort Farm	September 2013	Mackerel (28), Saithe (7)	rod and line	0
		July/August 2014	Mackerel (110), Whiting (41)	rod and line	0
Loch Leven	Leven Farm	July/August 2014	Mackerel (17), Whiting (55)	rod and line	0
Loch Melfort	Reference Mackerel	September 2013	Mackerel (22)	rod and line	~ 1
Oban Bay	Reference Saithe	October 2013	Saithe (7)	rod and line	~ 1
Isle of Luing	Reference Mackerel 1	August 2014	Mackerel (69)	rod and line	> 5
Oban bay	Reference Mackerel 2	August 2014	Mackerel (67)	rod and line	~ 3
Mallaig	Reference Mackerel 3	September 2014	Mackerel (45)	rod and line	> 10
Firth of Clyde	Reference Whiting 1	August 2014	Whiting (40)	bottom-trawling	> 10
North Minch	Reference Whiting 2	August 2014	Whiting (55)	bottom-trawling	> 10
Mallaig	Reference Whiting 3	September 2014	Whiting (50)	rod and line	> 10

3.6.3 Fish processing

All captured fish were immediately placed on ice and transported to the Institute of Aquaculture, University of Stirling. All fish were frozen at -20°C until processing. Fish caught during fieldwork of 2013 were processed on October 8, 29, 30, 31 and November 1, 2013. Processing of fish collected in 2014 took place on September 8, 11, 12, 15, 16, 18, 19, 30 and October 1, 7, 8, 2014.

Fish were defrosted prior to processing. Individual mass (g) and length (cm) was taken for all processed fish. Individual fish were dissected. Following dissection fish livers were weighed and stored for further analysis. Livers were used for lipid and fatty analysis for Chapter 4. Following the processing of fish in 2014, some of the left over muscle tissue samples for mackerel were used for another research project.

3.6.3.1 Stomach content analysis

Stomachs (from the oesophagus to the pyloric sphincter) were removed and stored in 70% ethanol. Stomachs of mackerel and saithe collected in 2013 were analysed within four weeks to determine dietary composition of fish next to cages and their counterparts. Stomachs of mackerel and whiting collected in 2014 were analysed between 10-12 weeks. Stomach contents were emptied, and prey items were categorized into pellets, invertebrates, fish and unknown. Frequency of occurrence (FO) was calculated using the formula:

$$\mathrm{FO} = \frac{\mathrm{J_i}}{\mathrm{P}} \times 100 \qquad (eq.3.1)$$

where J_i is the number of fish containing prey *i* and P is the number of fish with food in their stomachs (Hyslop 1980).

3.6.3.2 Condition indices

Fulton's condition index (FCI) was calculated using the formula:

$$\mathrm{FCI} = \frac{W}{L^3} \times 100 \qquad (eq.3.2)$$

where W = mass (g), L = length (cm). The hepatosomatic index (HSI) was calculated with the formula:

$$\text{HSI} = \frac{\text{Liver mass (g)}}{\text{Total mass (g)}} \times 100 \qquad (eq.3.3)$$

3.6.3.3 Lipid extraction and fatty acid methyl esters (FAMEs)

FAs are widely used biological markers in studying types of foods consumed (reviewed by Dalsgaard et al. 2003). FAs were used as biomarkers in Chapters 4 and 5. Samples of the muscle (flesh) and liver tissues were obtained from individual mackerel and juvenile saithe sampled in 2013. In 2014, only muscle samples were obtained from mackerel and whiting caught near and away from the sea cages. The livers from whiting collected in 2014 were too small and deteriorated very fast during processing and therefore it was not possible to use them for total lipid and FA analysis. The livers from mackerel collected in 2014 were stored at -80°C. The livers were to be used for another research project.

All tissue samples for this project were stored at -20°C for lipid and fatty acid analysis. Commercial pellets were also collected from fish farms (Loch Melfort and Loch Leven) and were analysed for total lipid and FA analysis.

Lipid and fatty acid analysis of fish tissues and artificial pellets sampled in 2013 were analysed within four weeks of fish sampling and fish tissues sampled in 2014 were analysed within 12 weeks. Lipids deteriorate in fish samples during frozen storage and particularly in fatty fish such as mackerel (Aubourg et al. 2005; Romotowska et al. 2016). However, the overall fatty acid levels in fish tissues of both mackerel and gadoids are assumed to be relatively stable during the frozen storage time in this research (e.g. Xing et al. 1993; Romotowska et al. 2016).

Limited resources did not allow the lipid and fatty acid analysis on all sampled fish in 2013 and 2014. Therefore, prior to the start of lipid and fatty acid extraction a number of fish tissue samples were selected at random from the freezers.

3.6.3.4 Total lipid extraction

Total lipids were extracted from diet, muscle and liver tissues of fish according to the method of Folch et al. (1957). In brief, total lipids were extracted from samples (~ 0.5 g) by homogenising in 20 volumes of chloroform:methanol (2:1, v/v) using Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) in a fume cupboard. Samples were left on ice for one hour followed by addition of 5 ml of 0.88% (w/v) potassium

chloride (KCl) to remove non-lipid impurities. Samples were centrifuged at 400 × g (1500 rpm Jouan C 412 bench centrifuge) for 5 minutes and the top layer (aqueous) was removed by aspiration. The bottom layer was transferred to pre-weighed tubes through prewashed (with chroloform:methanol 2:1) filter paper (Whatman no.1). The mass of lipids was determined gravimetrically after evaporation of solvent under stream of oxygen-free nitrogen (OFN) and overnight desiccation under vacuum. Lipids were re-dissolved in chloroform:methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) at a concentration of 10 mg/ml and stored under nitrogen at -20°C prior to FA analysis. All lipid extractions were done in duplicate. Percent lipid was calculated as follows:

$$\% \text{ Lipid} = \frac{\text{Mass Lipid (g)}}{\text{Mass Sample (g)}} \times 100 \qquad (eq.3.4)$$

3.6.3.5 Fatty acid methyl esters (FAME) preparation

FA methyl esters (FAME) were prepared from total lipids by acid-catalysed transesterification according to the method of Christie (1982) and extracted and purified as described by Tocher and Harvie (1988). Total lipids (100 µl) and 17:0 free FA standard (heptadecaenoic acid) at 10% of the total lipid (100 µl) were mixed and the solvent evaporated under nitrogen evaporator. Tolouene (1 ml) was added to dissolve neutral lipids followed by addition of 2 ml methylating reagent (1% (v/v) solution of sulphuric acid in methanol). After mixing, the tubes were incubated overnight (16 hours) in a hot block at 50°C. Following incubation, tubes were cooled to room temperature and 2 ml of 2% (w/v) $KHCO_3$ and 5 ml of iso-hexane:diethyl ether (1:1, v/v) + 0.01% (w/v) BHT were added, mixed and centrifuged at 400 x g for 2 minutes. The upper organic layer was transferred to another test tube and additional 5 ml of isohexane:diethyl ether (1:1, v/v) (no BHT) was added and same procedure repeated. The solvent was evaporated under nitrogen evaporator and FAMEs re-dissolved in 100 µl of iso-hexane.

FAMEs were purified by thin layer chromatography (TLC) plates (20 × 20 cm). FAMEs were loaded on the plates using Hamilton syringe (100 µl). The samples were loaded at 1.5 cm from the bottom of the plate. Samples were separated by about 1.2 cm and by 2 cm margin from the edges of the plate. Plates were chromatographed in iso-hexane:diethyl ether:acetic acid (90:10:1, v/v/v). To visualise the FAMEs the margins

from the edges of the plates were sprayed with 1% (w/v) iodine in chloroform followed by scraping marked areas into a tube using scalpel blade. FAMEs were eluted from the silica with 10 ml of iso-hexane:diethyl ether (1:1, v/v) + 0.01% (w/v) BHT followed by centrifugation. The solvent was transferred to a test tube and evaporated to dryness under nitrogen. Samples were transferred to glass vials in 1 ml of iso-hexane. FAMEs were stored under nitrogen at -20°C until further analysis.

FAMEs were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d. × 0.25 μm ZB-wax column (Phenomenex, Cheshire, UK), on-column injector and a flame ionization detector. Hydrogen was used as a carrier gas with initial oven thermal gradient 50°C to 150°C at 40°C/min to a final temperature of 230°C at 2°C/min. Individual FAME were identified by comparison of their retention times with known standards (heptadecanoic acid (17:0) (internal standard); marinol oil (reference standard); Supelco^{TM} 37-FAME mix (Sigma-Aldrich Ltd., Poole, UK)) and by reference to published data (Ackman 1980; Tocher and Harvie 1988). Data were collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A., Milan, Italy). Individual FA concentrations were expressed as percentages of the total content. All samples were analysed in duplicates to ensure precision of the method.

PART I: EMPIRICAL STUDIES

CHAPTER 4
FATTY ACID BIOMARKERS INDICATE EFFECTS OF A HALIBUT FARM IN MACKEREL AND SAITHE

4.1 Introduction

Anthropogenic activities provide readily available resources of food in many environments. Food production waste, dumps, fisheries discards, animal and vegetal remains in fish and agricultural farms, nutrients in sewage and more are exploited by various organisms (Oro et al. 2013). Spatial and temporal predictability of anthropogenic food resources allows species to benefit from the easy access (Oro et al. 2013). This leads to decrease in foraging times which is often reflected in improved biological condition, better reproductive performance and decrease in mortality rates (e.g. predation) (Bartumeus et al. 2010; Almaraz and Oro 2011; Oro et al. 2013). For example, some facultative scavengers or opportunistic species such as cockroaches, foxes, gulls, rats and other top terrestrial predators (e.g. coyote, red fox) take advantage of these predictable food resources which is evident in their high abundances (Oro et al. 2013; Newsome et al. 2015). In marine environments, fish discards represent a food source for many organisms across the entire food web including whales and seabirds to benthic organisms (Oro et al. 2013). Here, I focus on food subsidy provided by a halibut farm and subsequent biological changes in condition and fatty acid (FA) profiles in mackerel and saithe.

To detect aquaculture impacts on wild fish associated with fish farms a number of researchers have used biochemical tracers such as FAs which are a useful tool for analysing dietary items that are assimilated over time (see Chapter 2; Dalsgaard et al. 2003). Aquafeeds have higher levels of vegetable oils which is reflected in modified FA profiles in farmed fish (Bell et al. 1996, 2001; Naylor et al. 2009). Similarly, modified FA profiles have been reported in wild fish feeding on artificial waste feed from fish farms (see subsection 2.1.4, Chapter 2).

This study was an observational and experimental study with the following aims: 1) to evaluate whether mackerel and saithe consume waste feed and if this is reflected in changes in FA profiles of muscle and liver tissues, 2) to evaluate whether mackerel and saithe directly feeding on a readily available food resources (waste feed) from a fish farm results in improved biological condition.

4.2 Methods

Underwater video camera was used to observe fish around the sea cages (see Chapter 3, section 3.3). Fishes, were sampled between September 14 and 22, 2013 using baited rod and line next to sea cages and at a reference site at approximately 1 km from the nearest sea cages (Figure 4.1). Details of the farm site, sampling methodology and fish processing can be found in Chapter 3.

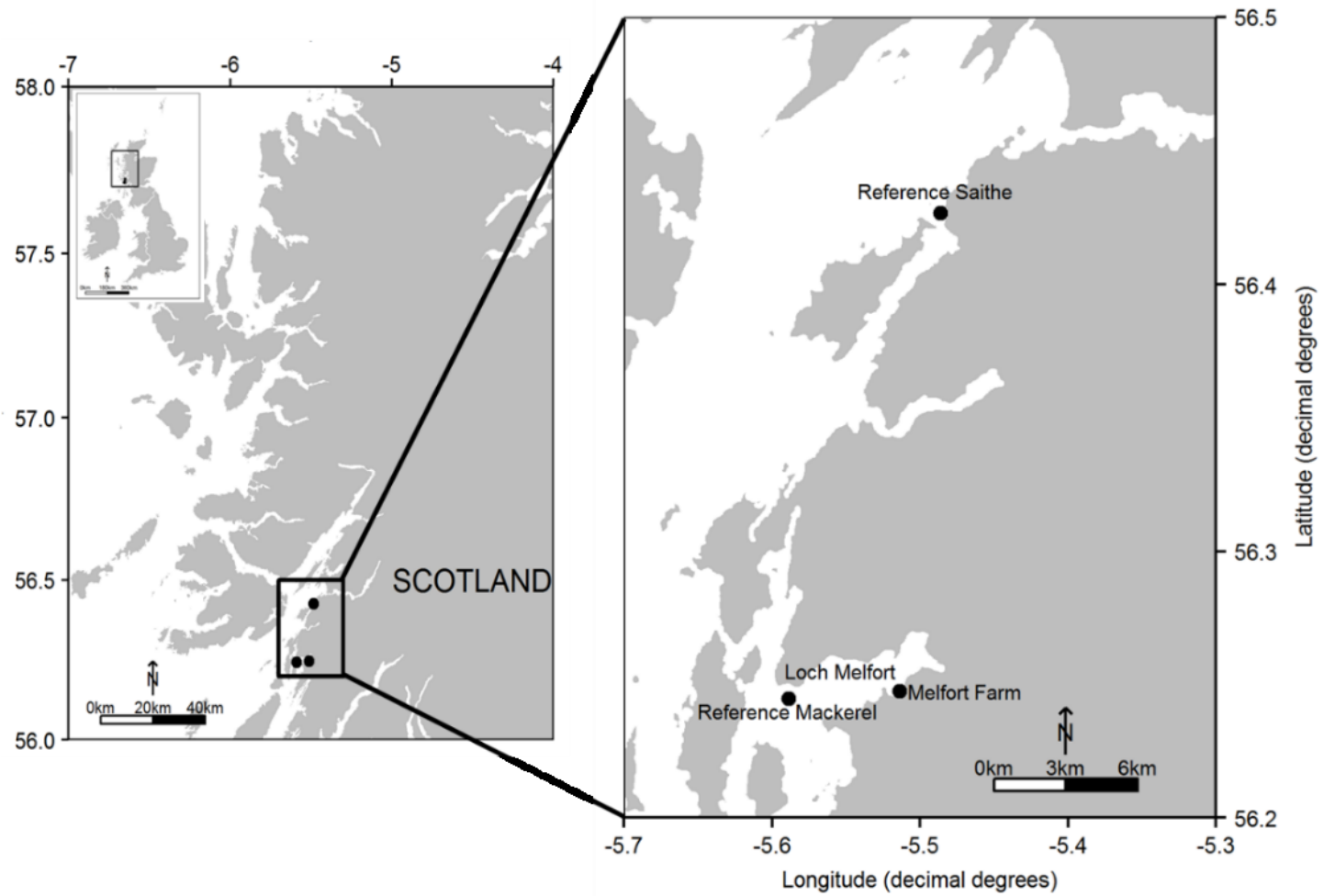

Figure 4.1 Sampling locations for mackerel and saithe near a halibut farm (Melfort Farm) and a reference site for each species (Reference Mackerel; Reference Saithe) on the West Coast of Scotland.

4.2.1 Statistical analysis

A range of univariate (parametric and nonparametric) and multivariate statistics were used to compare between FA profiles and biological status of fish caught near and away from cages. All statistical analysis was performed using the statistical software R (R Development Core Team 2016) run in RStudio (version 1.0.136, RStudio Team 2016).

4.2.1.1 Stomach content description

Frequency of occurrence (see Chapter 3) of each group of items (fish, fish pellets, invertebrates and unidentified) was calculated and plotted for both mackerel and saithe. Confidence intervals were estimated using the function binconf in library Hmisc (Harrell et al. 2016). The package plyr was also used for data arrangement (Wickham 2011).

4.2.1.2 Testing for differences in condition, lipids and total fatty acids between sites

The aim of the study was to establish whether there were any differences in length, mass, condition indices (FCI and HSI), lipid and fatty acid levels in mackerel and saithe sampled near one fish farm and compared to those sampled at a reference site.

Prior to applying any statistical models to the data a few graphical exploratory tools were used as suggested by Zuur et al. (2010). Boxplots were used to detect outliers or observations that are too far off from most of the observations. Both boxplots and a quantile-quantile (Q-Q) plots were used to get a general impression of the homogeinity and data distribution. Scatter plots were also applied to the data to explore relationships between variables. Scatter plots were drawn using tha package GGally (Schloerke et al. 2016).

A one way analysis of variance (ANOVA) models were applied to evaluate differences in length, mass, total lipid and selected individual fatty acid contents of mackerel and saithe caught near and away from sea cages. Analysis of covariance (ANCOVAs) models were applied to evaluate differences in FCI and HSI between the two groups of mackerel and saithe by taking into account length as a covariate. Length was used as a covariate in the analysis of FCI and HSI as it is often found as an important variable affecting the condition of fishes (see Richter et al. 2000; Lloret et al. 2002). Confidence intervals for all variables were estimated using the package lsmeans (Lenth 2016).

Results of the ANOVA models were considered valid if the assumptions of the models were generally met. The main assumptions of the model include normality and homogeneity of residuals (Underwood 1997). In addition to the main assumptions (normality and homogeneity of variance) of the ANOVA models, the ANCOVA model require that the relationship between the dependent variable and covariate to be linear and that there is homogeneity of regression slopes (Underwood 1997).

Multiple comparisons between similar parameters such as fatty acids needs to be corrected for because the probability of getting at least one significant result by chance is greater than the significance level of 0.05. Bonferroni correction, a common method used to correct for multiple comparisons, adjusts the p value at which a test is evaluated over the total number of tests being performed (Bonferroni 1936). In this study, a significance level with correction for multiple testing would be $\alpha = 0.05/15$ tests $= 0.003$. However, using Bonferroni correction can reduce the power to detect any effect and therefore it was not performed in this study (e.g. Cabin and Mitchell 2000; Moran 2003).

All lipid and fatty acid samples were duplicated to assess precision of the methodology. Thus, all duplicates were averaged prior to any analysis.

As the assumptions for the parametric models were violated, nonparametric models were also used for robustness against minor violations of ANOVA assumptions. The non-parametric Mann-Whitney U test assumes independence of observations and random sampling (Wilcox 2003). Mann-Whitney U test was used to test for statistical differences in some of the variables of mackerel and saithe sampled near and away from the fish farm.

4.2.1.3 Multivariate analysis of FAs

Of the 33 identified fatty acids (FAs), 15 fatty acids were selected based on the abundance and/or importance (14:0, 16:0, 18:0; 16:1n-7; 18:1n-7; 20:1n-9; 22:1n-11, 20:4n-6, 18:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3) and potential aquaculture biomarkers (18:2n-6, 18:3n-3 and 18:1n-9) (Iverson 2009).

Principal component analysis (PCA) was used as an exploratory technique to describe the relationship among samples. The aim of this technique is to reduce a large number of variables into a new set of variables (principal components) which is a linear combination of the original variables. Some of the main assumptions of the PCA include linearity between variables, principal components with large variances are of more

interest than those with lower variances, and orthogonality of the principal components (e.g. Shlens 2003).

To visualize the correlation between original variables (FA proportions) and the samples a biplot was drawn. The closer two observations are to each on the biplot the more similar their FA composition. Correlations between two variables is also indicated by the angle of the lines connecting the two variables. If the angle between two variables is 0 degrees then the variables are highly correlated, if the angle is 180 degrees there is negative correlation and a 90 degree angle indicates no correlation. The arrows or loadings displayed on the biplot is the correlation between the original variable and the new variable which indicate the direction and magnitude in which the variable increases (Budge et al. 2006; Everitt and Hothorn 2011). PCA was run using the built-in function prcomp.

4.3 Results

4.3.1 Observations (anecdotal accounts)

All fishes observed with the underwater video equipment and sampled as described in Chapter 3 can be found in Appendix A. Based on observations and sampling relatively more marine organisms (fishes and benthic organisms) were noted near the sea cages than at the reference sites (Appendix A). Pelagic (mackerel and clupeids) and benthopelagic/benthic (gadoids, flatfishes) fishes were noted near the fish farm (Appendix A). Very small fish (~1-2 cm) were noted around some of the cages. Benthic organisms near the fish farm included polychaetes, echinoderms, crabs and lobsters (see Appendix A). Seabirds during all the visits were noted near the fish farm. One seal was noted only on one occasion near the sea cages. A common skate was also caught and released during one of the visits. The underwater video recordings also revealed mackerel schools feeding on clupeids (Figure 4.2) and artificial feed (Figure 4.3) besides the sea cages. More details on the species noted near the sea cages can be found in Appendix A.

Figure 4.2 Mackerel feeding on juvenile clupeids next to sea cages (see https://www.youtube.com/watch?v=6q_5zBQGKoU).

Figure 4.3 Mackerel feeding on waste pellets lost through sea cages (see https://www.youtube.com/watch?v=IkVr5IDMnKQ).

4.3.2 Scatter plots

Relationship patterns between various variables (length, mass, FCI, HSI and total lipids) for mackerel (Figure 4.4) and saithe (Figure 4.6) were evaluated using scatter plots near and away from the sea cages. Scatter plots were also used to evaluate the relationship between condition and selected FAs for mackerel (Figure 4.5) and saithe (Figure 4.7). It is worth noting that the scatter plots are only for those mackerel that were used for lipid and fatty acid analysis.

4.3.2.1 Mackerel

Length was found positively correlated with mass for mackerel ($r = 0.97$, $p < 0.000$) (Figure 4.4). FCI was positively correlated with length ($r = 0.73$, $p < 0.000$) and mass ($r = 0.85$, $p < 0.000$) whereas HSI was negatively correlated with length ($r = -0.47$, $p < 0.01$ and mass ($r = -0.41$, $p < 0.01$) (Figure 4.4). Total lipids were positively correlated with FCI ($r = 0.69$, $p < 0.000$) (Figure 4.4). HSI was found negatively correlated with FAs 18:2n-6 ($r = -0.59$, $p < 0.001$), 18:3n-3 ($r = -0.52$, $p < 0.01$), and positively correlated with n-3/n-6 ratio ($r = 0.61$, $p < 0.001$) (Figure 4.5). Both FAs 18:2n-6 and 18:3n-3 were negatively correlated with the n-3/n-6 ratio (Figure 4.5).

Overall the scatter plots indicated that some mackerel near the sea cages were longer, heavier, have higher FCI and more lipid in muscle tissues than those sampled away from the farm (Figure 4.4). Additionally, some mackerel sampled near the sea cages have higher FCI and low n-3/n-6 ratio when compared to those sampled from a reference site. Some mackerel caught near sea cages have an overall lower HSI and lower n-3/n-6 ratio than mackerel sampled away from cages (Figure 4.5). It is also worth noting that there is a higher variability in the different variables of mackerel sampled near the cages than those sampled away.

4.3.2.2 Saithe

Length was positively correlated with mass ($r = 0.92$, $p < 0.000$) (Figure 4.6). Total lipid content in muscle tissues was negatively correlated with FCI ($r = -0.53$, $p < 0.01$) and HSI ($r = -0.56$, $p < 0.01$) (Figure 4.6). FCI was positively correlated with FAs 18:2n-6 ($r = 0.67$, $p < 0.001$) and negatively correlated with n-3/n-6 (Figure 4.7). HSI was positively correlated with FA 18:2n-6 ($r = 0.78$, $p < 0.000$) (Figure 4.7). Both FAs, 18:2n-6 and 18:3n-3, were negatively correlated with n-3/n-6 ratio (Figure 4.7).

Overall saithe sampled next to the sea cages had higher FCI, HSI and lower total lipid content in muscle tissues than those sampled from a reference site. Saithe near cages that are higher in FCI and HSI also have higher contents of FAs 18:2n-6 and 18:3n-3 and lower n-3/n-6 ratios (Figure 4.7).

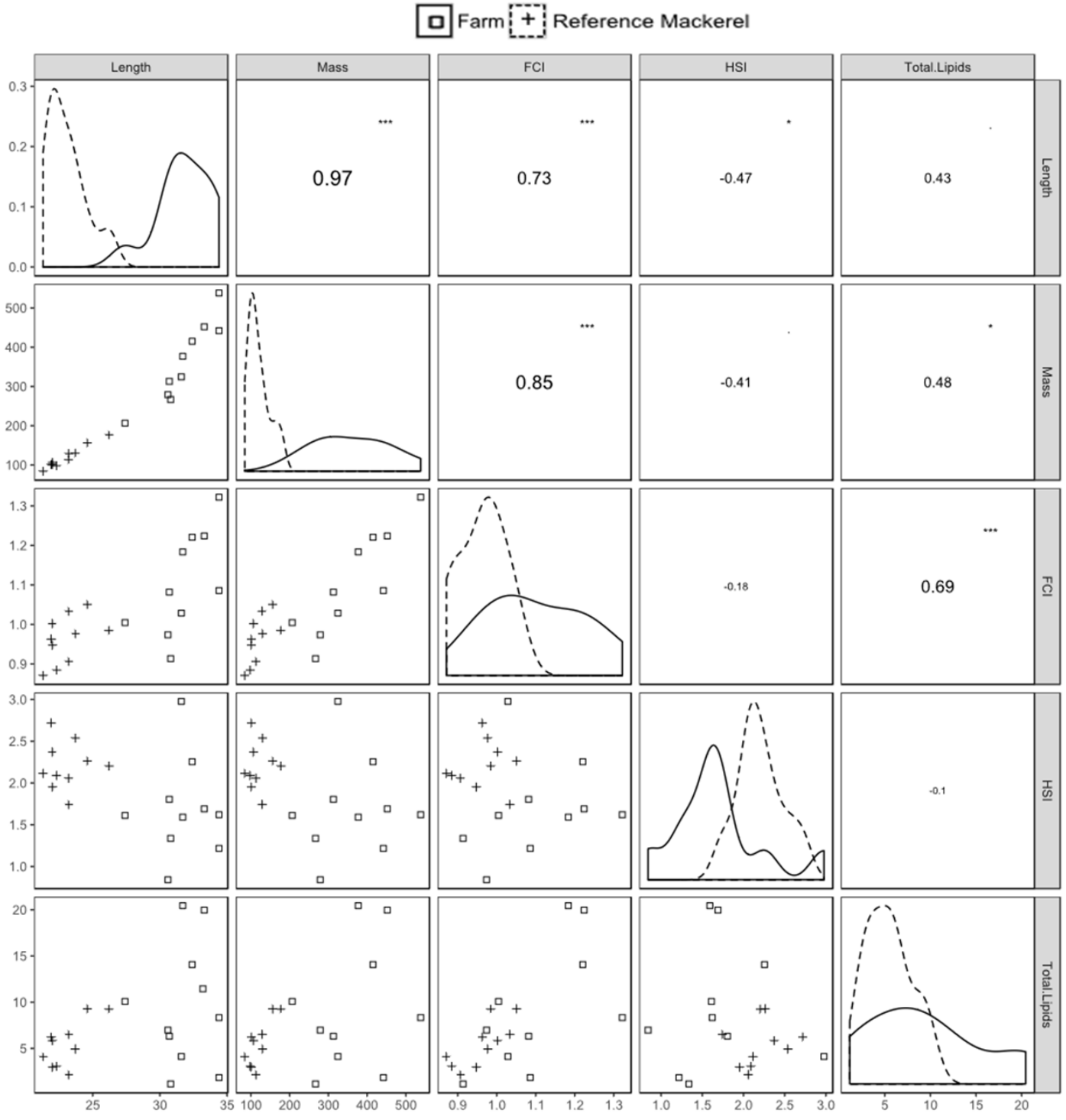

Figure 4.4 Scatter plots of length (cm), mass (g), FCI, HSI, and total lipid contents (%) in muscle of mackerel caught near and away from a halibut farm. Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

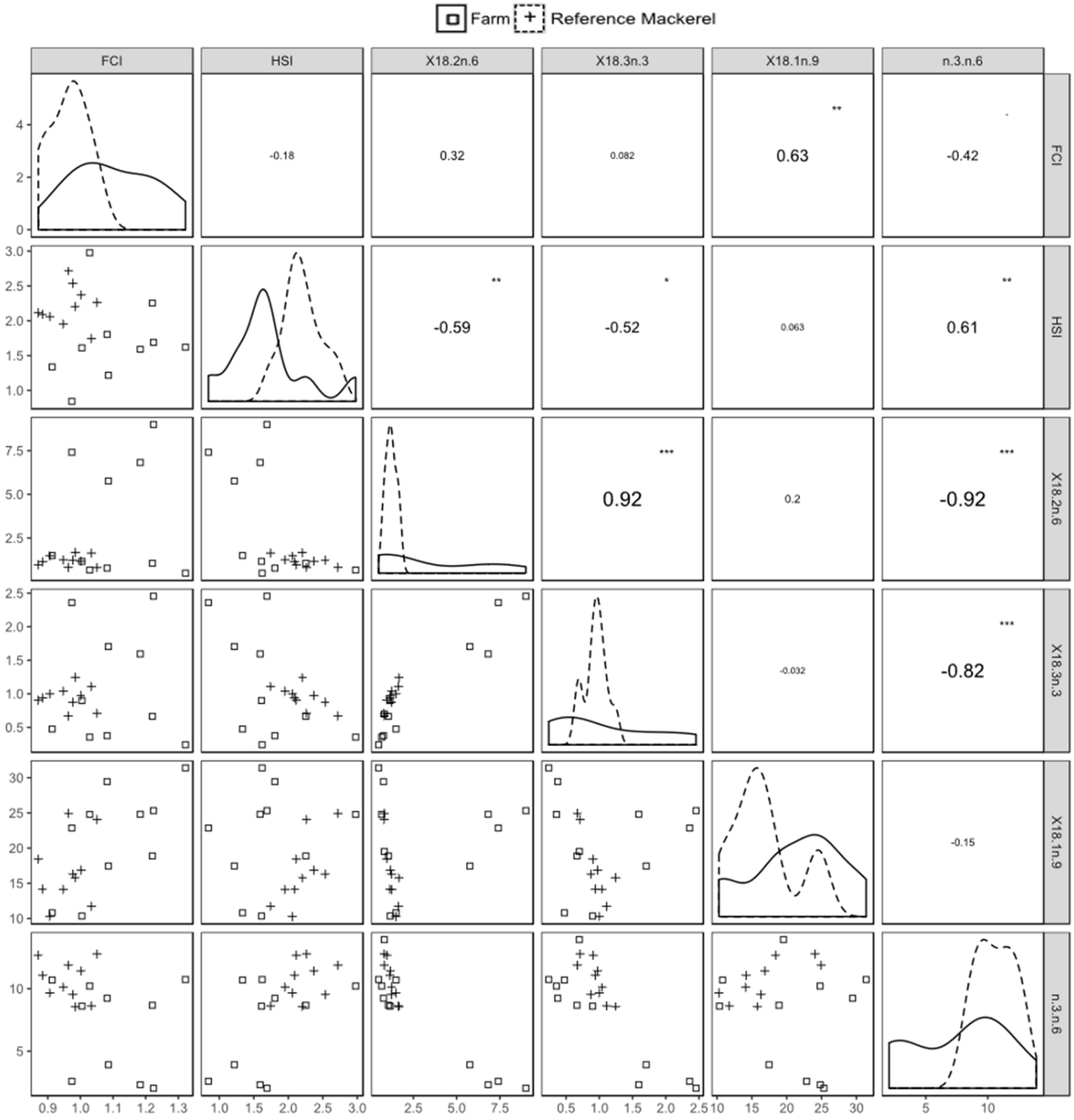

Figure 4.5 Scatter plots of FCI, HSI, and selected FAs (18:2n-6, 18:3n-3, 18:1n-9) and n-3/n-6 ratio in the muscle of mackerel caught near and away from a halibut farm. Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

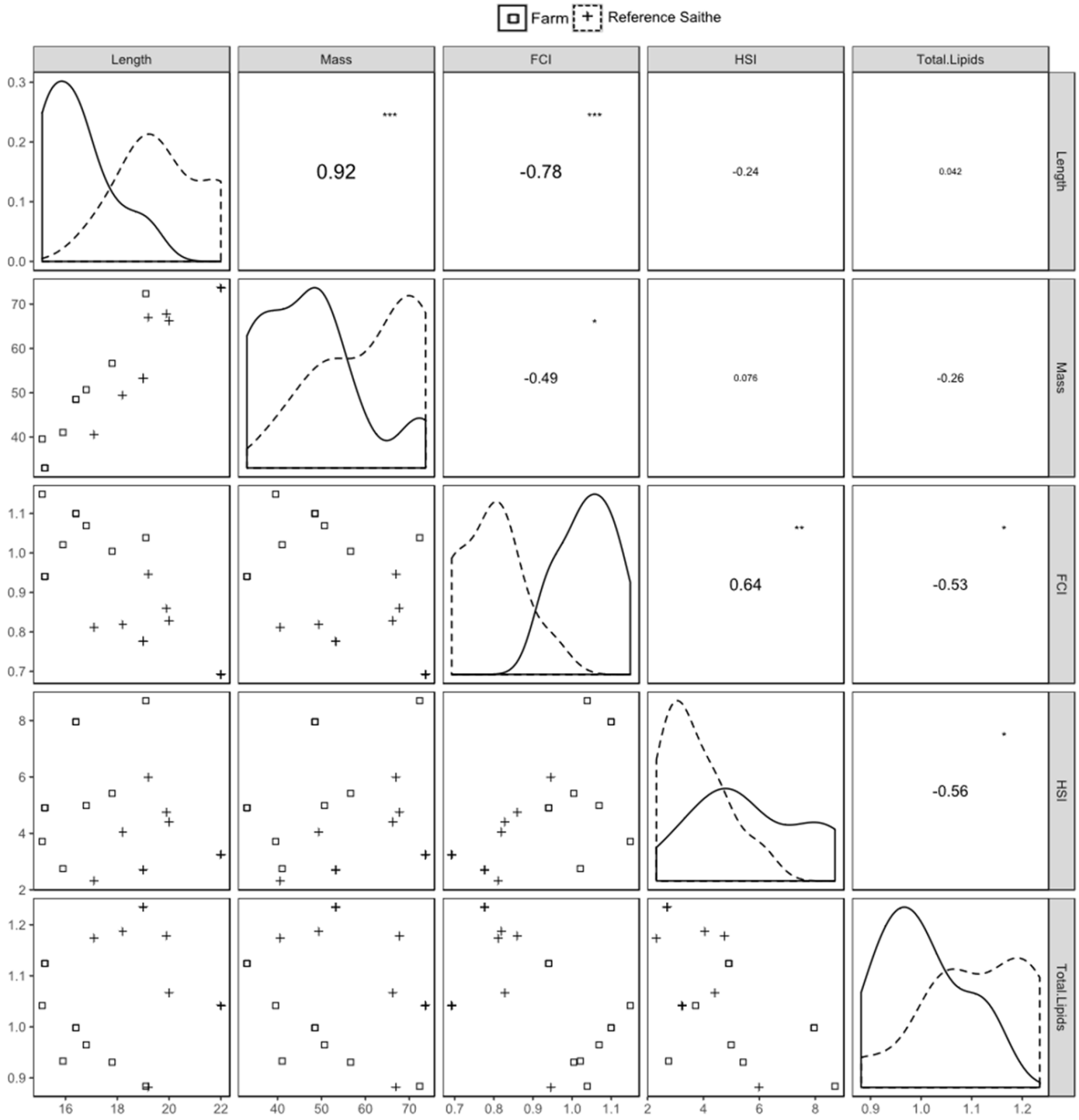

Figure 4.6 Scatter plots of length (cm), mass (g), FCI, HSI, and total lipid content (%) in muscle of saithe caught near and away from a halibut farm. Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

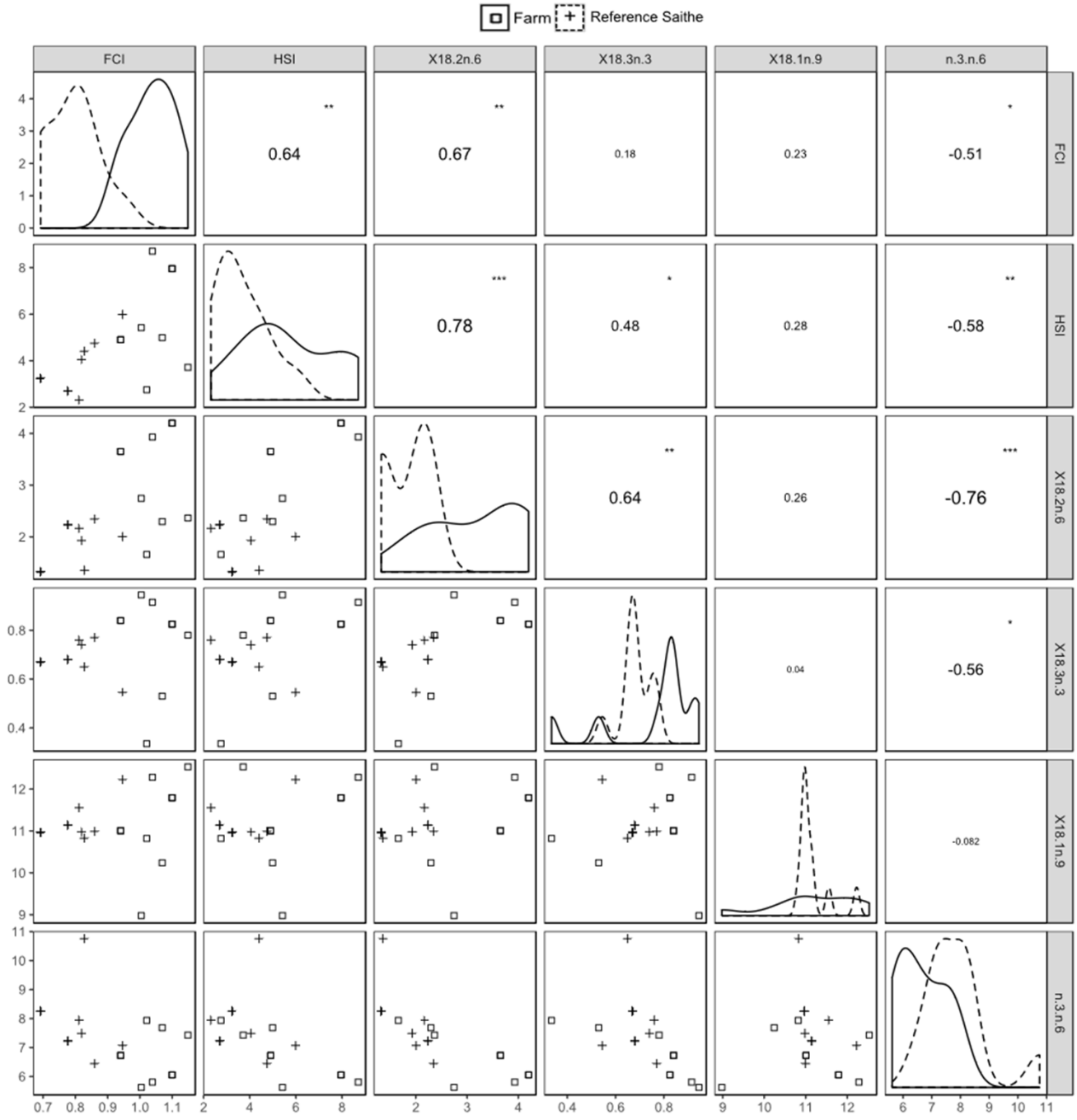

Figure 4.7 Scatter plots of FCI, HSI, and selected FAs (18:2n-6, 18:3n-3, 18:1n-9) and n-3/n-6 ratio in the muscle of saithe caught near and away from a halibut farm. Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

4.3.3 Stomach contents

Stomach content analysis for both mackerel and saithe is presented in Figure 4.8 A and B, respectively. Stomach content analysis was performed on all fish reported in Table 4.1.

4.3.3.1 Mackerel

The majority of the mackerel caught near and away from the sea cages preyed on clupeids. Waste feed was found in 31% of the mackerel caught next to the sea cages and never in mackerel away from cages. Mackerel caught away from cages had more empty stomachs (36%) than those caught near the farm (7%) (Figure 4.8A).

4.3.3.2 Saithe

Clupeids were the main item found in 71% of the saithe caught near the sea cages. Waste feed was found in 29% of the saithe associated with sea cages and never in saithe away from cages. All saithe caught away from cages had invertebrates (e.g. periwinkles, shrimp, polychaetes) in their stomachs. None of the invertebrates found in the stomach of saithe were identified to a taxonomic level. All fish caught near the fish farm had full stomachs and 43% of the saithe caught at reference site were empty (Figure 4.8B).

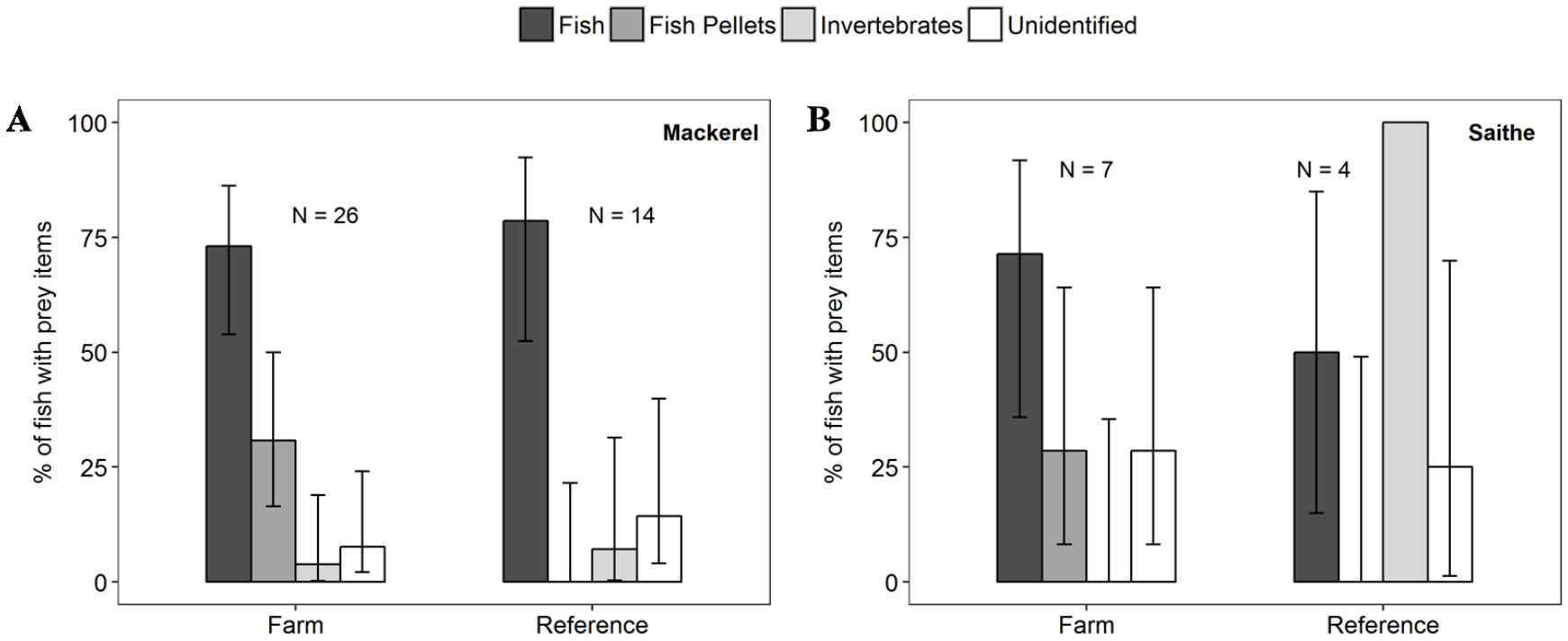

Figure 4.8 Stomach contents of mackerel (A) and saithe (B) caught near a fish farm and at reference sites. Bars are drawn with 95% confidence intervals. N is the number of fish with non-empty stomachs.

4.3.4 Testing for differences in length, mass and condition

4.3.4.1 Mackerel

Summary of the length, mass, and condition indices (FCI and HSI) for both mackerel and saithe near and away from cages are presented in Table 4.1. Results of the ANOVA/ANCOVA models for all variables can be found in Table 4.2. The residual analysis for all the ANOVA/ANCOVA models can be found in Appendix B. The results of the Mann-Whitney U tests for the different variables are presented in the subsections of mackerel and saithe.

Some groups show greater variance in measurements (e.g. Figure B.1, length and mass) but this appears to reflect the fact that some fish in the farm groups had more exposure to the impact of the farm resulting in a bimodal distribution for that group. There is no measurement in the data set that would allow the two groups within the bimodal distribution to be modelled separately. Thus, non-parametric tests were used along with the parametric models.

Length and mass of mackerel sampled near the sea cages were statistically different than the length and mass of mackerel sampled at the reference site (Table 4.1)(ANOVA, Reference vs. Farm length difference = -7.76, 95% CI: [-9.44, -6.08], $F = 86.39$, $p < 0.000$; ANOVA, Reference vs. Farm mass difference = -202.10, 95% CI: [-251.79, -152.40], $F = 66.94$, $p < 0.000$).

Because of possible bimodality, nonparametric models were also used to confirm differences in length and mass. Using the Mann-Whitney U tests indicated statistically significant differences in the median length and mass of mackerel sampled near and away from the sea cages (Farm vs. Reference median length difference: 8.40, 95% CI: [7.00, 9.80], $W = 588.5$, $p < 0.000$) and Farm vs. Reference median mass difference: 213.9, 95% CI: [160.9, 259.9], $W = 567$, $p < 0.000$).

No statistically significant differences were found for FCI (ANOVA, Reference vs. Farm difference: -0.017, 95% CI: [-0.069, 0.034], $F = 1.25$, $p = 0.27$) and HSI (ANOVA, Reference vs. Farm difference: 0.041, 95% CI: [-0.24, 0.32], $F = 0.239$, $p = 0.627$) of mackerel near and away from the farm (Table 4.2). Based on the diagnostic plots for the parametric models (see Figure B.1) the normality and the homogeneity of variances were acceptable and therefore nonparametric models were not run.

4.3.4.2 Saithe

Statistical differences were found in length (ANOVA, Reference vs. Farm difference: 2.73, 95% CI: [0.99, 4.47], F = 11.69, p = 0.0051), FCI (ANOVA, Reference vs. Farm difference: -0.10, 95% CI: [-0.18, -0.01], F = 12.07, p = 0.0052) and HSI (ANOVA, Reference vs. Farm difference: -1.59, 95% CI: [-5.80, -0.46], F = 6.678, p = 0.0254) between saithe caught near the sea cages and at a reference site (Table 4.2). No statistical differences were found between the mass (ANOVA, Reference vs. Farm difference: 10.87, 95% CI: [-3.73, 25.47], F = 2.63, p = 0.131) of saithe near and away from cages (Table 4.2).

The diagnostic plots of the parametric models for HSI indicated slight violations of the model assumptions (e.g. Figure B.2) such as deviation from normality and lack of homogeneity of variances. These deviations did not appear to be strong and therefore no equivalent non-parametric models were used. As with the data of mackerel no values in the data of saithe were excluded as outliers. Some values showed some variation from the groups (e.g. Figure B.2, (length, mass, HSI)) but this appears to reflect the fact that some fish had more exposure to the impact of the farm that should be incorporated in the model, rather than an incorrect measurement or an outlier that should be removed.

Table 4.1 Number of fish, length, mass, Fulton's condition index (FCI) and hepatosomatic index (HSI) for mackerel and saithe caught next to and away from a halibut farm in Loch Melfort. 95% confidence interval estimates of the sample means are presented.

	Mackerel		Saithe	
	Farm	**Reference Mackerel**	**Farm**	**Reference Saithe**
No. of fish	28	22	7	7
Length (cm)ǂ	30.1 [28.5, 31.4]	22.3 [21.5, 23.1]	16.6 [15.3, 17.9]	19.3 [17.9, 20.8]
Mass (g)	310 [264, 357]	108 [96, 120]	49 [36.8, 60.8]	60 [48.6, 70.8]
FCI#	1.10 [1.05, 1.14]	0.95 [0.92, 0.99]	1.05 [0.98, 1.11]	0.82 [0.75, 0.89]
HSI# (%)	1.77 [1.57, 1.97]	2.00 [1.79, 2.21]	5.49 [3.51, 7.48]	3.92 [2.74, 5.10]

ǂ Length is fork length (cm) for mackerel and total length (cm) for saithe. #FCI=Mass (g)/(Length (cm))^3 * 100; HSI=Mass of liver (g)/Mass (g) *100

Table 4.2 Results of the analysis of variance (ANOVA) models for length, mass, Fulton's condition index (FCI) and hepatosomatic index (HSI) for mackerel and saithe caught next to and away from cages. An ANCOVA model was applied to the FCI and HSI data. ***Note**:* Df: degrees of freedom, Sum Sq: Sum of squares, Mean Sq: Mean of squares. Significance level: $P < 0.05$.

	Mackerel					Saithe				
Length										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	741.7	741.7	86.39	0.0000	1	26.06	26.058	11.69	0.0051
Residuals	48	412.1	8.6			12	26.75	2.229		
Mass										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	495110	495110	66.94	0.0000	1	413.3	413.3	2.63	0.131
Residuals	47	347640	7397			12	1885.9	157.2		
FCI										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Length	1	0.2958	0.2958	37.14	0.0000	1	0.1208	0.1208	22.68	0.0006
Treatment	1	0.0099	0.0099	1.25	0.27	1	0.0643	0.0643	12.07	0.0052
Residuals	46	0.3663	0.0080			11	0.0586	0.0586		
HSI										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Length	1	0.681	0.6807	2.927	0.094	1	0.002	0.002	0.001	0.9791
Treatment	1	0.056	0.0555	0.239	0.627	1	17.382	17.382	6.678	0.0254
Residuals	45	10.464	0.2325			11	28.631	2.603		

4.3.5 Total lipids and fatty acid profiles

The lipid and FA composition of the diet used in Loch Melfort can be found in Table 4.3. The lipid and fatty acid (FA) composition of mackerel and saithe caught near and away from cages are presented in Tables 4.4 and 4.5, respectively. Full FA profiles for diets, mackerel and saithe can be found in Appendix B. The results of the ANOVA models for total lipid and fatty acids analysis tissues can be found in Table 4.6 for mackerel and Table 4.7 for saithe. The diagnostic plots for all the models used to analyse the data can be found in Figures B.3, B.4. Additionally, because of possible violation of the model assumptions (e.g. normality and homogeneity of variances) for ANOVA, non-parametric tests (Mann-Whitney U-tests) were used for some variables for both mackerel and saithe.

4.3.5.1 Commercial diet composition

Halibut was fed a diet specially formulated for the species. The diet information was obtained from the staff at the farm. The analytical constituents of the diet were: lipids (24.0%), protein (43%), ash (6.2%), fibre (2.8%), calcium (1.0%), phosphorus (1.2%) and sodium (0.4%). The composition of the diet was: fish meal, fish oil, vital wheat gluten, horse beans dehulled, maize gluten, sunflower seed expeller, soya (bean) meal, mono-ammonium phosphate, lysine, vitamins and minerals. The commercial feed had a total lipid level of 21.19% (Table 4.3). The total n-6 PUFAs were lower than the total n-3 PUFAs which was also reflected in high n-3/n-6 ratio (Table 4.3). The overall total PUFA levels were higher than the total SFAs and total MUFAs (Table 4.3).

4.3.5.2 Mackerel

The three most abundant FAs in both muscle and liver tissues were 16:0, 18:1n-9 and 22:6n-3 (Table 4.4). No statistical differences were found in muscle and liver lipid contents of mackerel caught near and away from the sea cages by ANOVA. (Tables 4.4 and 4.6). Saturated fatty acids (SFAs) and n-3/n-6 ratios in both muscle and liver tissues were statistically different between mackerel caught near and away from sea cages (Table 4.6).

The diagnostic plots for some of the fatty acids indicated that some of the assumptions (e.g. normality and homogeneity of variances) were violated (see Figure

B.3). For example, the diagnostic plots for the muscle FAs 14:0, 16:0, 18:0, SFA, 16:1n-7, 18:1n-7, 20:1n-9, 22:1n-11, MUFAs, 18:2n-6, n-6 PUFAs, 18:3n-3, 20:5n-3, 22:6n-3, total PUFAs and n-3/n-6 showed some lack of homogeneity of variances and some values that appear to be further apart from the rest of the data points (Figure B.3). Similarly, some lack of homogeneity of variances and data points with higher variance than the rest of the data were noted for the liver FAs 16:0, SFAs, 16:1n-7, 18:1n-9, 18:1n-7, 22:1n-11, 18:2n-6, 20:4n-6, n-6 PUFAs, 18:3n-3, 22:5n-3, 22:6n-3, n-3 PUFAs, total PUFAs, n-3/n-6 (Figure B.3).

No values in the data were excluded as outliers. Some values show substantial variation from the groups (e.g. Figure B.3) but this appears to reflect the fact that some fish had more exposure to the impact of the farm that should be incorporated in the model, rather than an incorrect measurement or an outlier that should be removed.

Equivalent non-parametric tests (Mann-Whitney U-tests) were used as remedy for the violation of assumptions (deviations from normality and heterogeneity of variances) in the parametric models. The Mann-Whitney U tests indicated statistically significant differences in SFAs in muscle tissues and n-3/n-6 in liver tissues in mackerel sampled near and away from cages (Table 4.8). The n-3 PUFAs in muscle tissues were also statistically different between mackerel sampled near and away from the sea cages (Table 4.8).

The principal component analysis (PCA) for the FAs of muscle and liver of mackerel near and away from cages can be found in Figures 4.9A and 4.9B, respectively. Two of the principal components (PC1 and PC2) explained 66.1% of the total variation of FA in muscle samples (Figure 4.9A). Principal component 1 mainly comprised the variations in 20:1n-9, 22:1n-11, 14:0, 18:2n-6, 18:3n-3 and 16:0, 18:0 (the latter two with negative correlation) while variations in 22:5n-3, 20:5n-3, 20:4n-6, 22:6n-3, and 18:1n-9 were contained by PC2 (Figure 4.9A). A combination of two principal components (PCs) explained 70.5% of the total variation of FA profiles in liver samples (PC1: 55.7%, PC2: 14.8%) (Figure 4.9B). Variations mainly in 22:1n-11, 18:2n-6, 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3 and with negative correlation in 16:0, 18:0 and 18:1n-9 among liver samples were explained by PC1 while PC2 contained variations of 18:1n-7 and 16:1n-7 (Figure 4.9B).

4.3.5.3 Saithe

The four most abundant FAs in both tissues were 16:0, 18:1n-9, 20:5n-3 and 22:6n-3 (Table 4.5). No statistical differences were found in total lipid content of muscle and liver tissues of saithe caught near and and away from sea cages using the ANOVA models (Tables 4.5 and 4.7). The FAs 14:0, 18:2n-6, and 22:5n-3 in both muscle and liver tissues were statistically different (ANOVA) between saithe caught near and away from the farm (Table 4.7). Using the ANOVA models, statistical differences in n-3/n-6 ratios were found between liver tissues in saithe sampled near and away from the sea cages (Table 4.7).

The diagnostic plots for the individual FAs indicated some violations in the assumptions of the ANOVA models (Figure B.4). For example, some lack of homogeneity of variances and some observations that deviate from the rest of the data were noted in the muscle FAs 14:0, 16:0, 16:1n-7, 18:1n-9, 18:1n-7, MUFAs, 20:4n-6, 18:3n-3, total PUFAs, and n-3/n-6 (Figure B.4). Similarly, lack of homogeneity of variances and points that appear to be further from the rest of the data were noted for the liver FAs 16:0, SFAs, 18:1n-9, 20:5n-3, 22:5n-3, total n-3 PUFAs, total PUFAs, and n-3/n-6 (Figure B.4).

As with the data for mackerel no values were excluded as outliers. Although some values indicate substantial variation from the groups this appears to reflect the fact that some fish had more exposure to the impact of the farm that should be incorporated in the model, rather than an incorrect measurement or an outlier that should be removed.

Some non-parametric tests (Mann-Whitney U test) were run to remedy some of the assumptions that were violated in the parametric tests. The results of the Mann-Whitney U tests indicated statistical differences between both saithe muscle and liver tissues sampled near and away from cages (Table 4.9). Using the Mann-Whitney U tests statistical differences were found for the FAs 18:2n-6, 22:5n-3 and n-3/n-6 ratio between muscle tissues of saithe sampled near and away from the farm (Table 4.9).

A combination of the two principal components (PCs) explained 55.0% of the total variation of FA profiles in muscle samples (PC1: 34.6%, PC2: 20.4%) (Figure 4.10A). Variations in 18:2n-6, 22:1n-11, 14:0 and 22:6n-3 among muscle samples were explained by PC1 while PC2 explained variations of 20:5n-3, 18:1n-7, and 16:0 (Figure 4.10A). A combination of the two principal components (PCs) explained 50.5% of the total variation of FA profiles in liver samples (PC1: 28.9%, PC2: 21.6%) (Figure 4.10B). Variations

mainly in 22:6n-3, 22:5n-3 and 18:1n-9, 20:1n-9, 20:4n-6 (negative correlation in the latter three) among liver samples were explained by PC1 while PC2 explained variations mainly of 18:2n-6, 18:3n-3, 16:0 and 18:0.

Table 4.3 Total lipid content (%) and fatty acid composition (%) of commercial diet used at Melfort farm. 95% confidence interval estimates of the sample means are presented.

	Diet
Total Lipid	21.19 [21.16, 21.21]
Fatty Acids	
14:0	7.09 [6.77, 7.40]
16:0	18.35 [16.83, 19.87]
18:0	3.66 [3.28, 4.04]
Total SFAs	30.02 [28.06, 31.99]
16:1n-7	7.64 [6.30, 8.97]
18:1n-9	12.94 [12.11, 13.76]
18:1n-7	2.77 [2.45, 3.08]
20:1n-9	1.74 [1.68, 1.81]
22:1n-11	2.10 [1.72, 2.48]
Total MUFAs	28.32 [25.78, 30.86]
18:2n-6	7.22 [7.03, 7.42]
20:4n-6	0.97 [0.90, 1.03]
Total n-6 PUFAs	8.95 [8.37, 9.52]
18:3n-3	1.09 [0.89, 1.28]
18:4n-3	2.11 [1.86, 2.36]
20:5n-3	13.56 [12.29, 14.83]
22:5n-3	1.70 [1.38, 2.01]
22:6n-3	9.58 [7.67, 11.49]
Total n-3 PUFAs	28.66 [24.58, 32.72]
Total PUFAs	41.66 [37.14, 46.17]
n-3/n-6	3.20 [2.95, 3.45]

Table 4.4 Total lipid (%) and fatty acid composition (%) of muscle and liver of mackerel caught near and away from a halibut farm. 95% confidence interval estimates of the sample means are presented.

	Muscle		Liver	
	Farm	**Reference Mackerel**	**Farm**	**Reference Mackerel**
No. of fish	11	10	11	10
Total Lipid	9.72 [6.04, 13.4]	5.43 [3.65, 7.21]	12.14 [9.81, 14.47]	10.52 [9.64, 12.40]
Fatty Acids				
14:0	2.75 [1.65, 3.86]	3.22 [2.68, 3.77]	0.60 [0.43, 0.77]	0.55 [0.43, 0.67]
16:0	17.83 [16.24, 19.42]	19.02 [18.36, 19.68]	18.36 [15.96, 20.75]	21.13 [19.86, 22.40]
18:0	4.89 [4.06, 5.71]	5.19 [4.76, 5.63]	5.21 [4.24, 6.18]	6.14 [5.58, 6.70]
Total SFAs	26.23 [24.66, 27.80]	28.47 [27.82, 29.12]	24.60 [21.40, 27.80]	28.33 [26.75, 29.91]
16:1n-7	4.00 [3.39, 4.62]	4.08 [3.64, 4.52]	3.11 [2.56, 3.65]	3.01 [2.53, 3.48]
18:1n-9	21.43 [16.84, 26.01]	16.67 [13.26, 20.08]	37.69 [33.21, 42.16]	39.64 [35.42, 43.86]
18:1n-7	4.35 [3.60, 5.09]	4.39 [3.96, 4.81]	7.47 [6.60, 8.33]	7.24 [6.69, 7.79]
20:1n-9	3.84 [2.79, 4.89]	3.30 [2.74, 3.86]	3.85 [3.39, 4.30]	3.17 [2.54, 3.79]
22:1n-11	4.25 [1.98, 6.51]	4.07 [2.87, 5.26]	1.72 [0.73, 2.72]	0.56 [0.34, 0.77]
Total MUFAs	40.48 [35.33, 45.62]	35.19 [32.05, 38.32]	56.27 [52.50, 60.04]	55.70 [51.66, 59.73]
18:2n-6	3.29 [1.02, 5.43]	1.22 [1.00, 1.44]	2.69 [0.46, 4.08]	0.51 [0.19, 0.83]
20:4n-6	1.04 [0.82, 1.26]	1.01 [0.88, 1.15]	0.96 [0.66, 1.27]	0.69 [0.51, 0.87]
Total n-6 PUFAs	5.13 [2.94, 7.33]	3.13 [2.68, 3.59]	4.03 [1.61, 6.45]	1.63 [0.86, 2.40]
18:3n-3	1.08 [0.53, 1.62]	0.95 [0.82, 1.07]	0.57 [0.14, 1.00]	0.21 [0.07, 0.35]
18:4n-3	1.15 [0.78, 1.53]	1.76 [1.50, 2.01]	0.19 [0.10, 0.28]	0.13 [0.07, 0.21]
20:5n-3	6.88 [6.08, 7.68]	8.31 [7.51, 9.11]	3.12 [2.38, 3.86]	2.62 [1.95, 3.28]
22:5n-3	1.75 [1.58, 1.91]	1.71 [1.57, 1.86]	2.07 [1.03, 3.11]	1.46 [0.62, 2.31]
22:6n-3	15.91 [11.16, 20.66]	18.93 [17.16, 20.69]	7.84 [6.33, 9.34]	8.74 [7.17, 10.31]
Total n-3 PUFAs	27.65 [22.58, 32.23]	32.72 [29.90, 35.11]	14.37 [10.65, 18.08]	13.61 [10.26, 16.95]
Total PUFAs	33.29 [28.40, 38.19]	36.34 [33.39, 39.30]	19.12 [13.11, 25.14]	15.97 [11.66, 20.28]
n-3/n-6	7.54 [4.78, 10.30]	10.63 [9.52, 11.75]	5.56 [3.51, 7.61]	9.68 [7.51, 11.85]

Table 4.5 Total lipid (%) and fatty acid composition (%) of fish muscle and liver of saithe caught near and away from a halibut farm in Loch Melfort. 95% confidence interval estimates of the sample means are presented.

	Muscle		Liver	
	Farm	**Reference Saithe**	**Farm**	**Reference Saithe**
No. of fish	7	7	7	7
Total Lipid	0.98 [0.94, 1.07]	1.11 [1.05, 1.19]	47.17 [42.28, 52.05]	46.47 [40.21, 54.35]
Fatty Acids				
14:0	1.28 [1.01, 1.54]	0.94 [0.84, 1.03]	2.50 [1.99, 3.00]	1.98 [1.76, 2.20]
16:0	17.72 [17.29, 18.16]	17.02 [16.62, 17.43]	14.68 [13.93, 15.44]	15.44 [14.61, 16.27]
18:0	5.65 [5.37, 5.92]	6.39 [6.13, 6.64]	6.51 [5.74, 7.28]	6.27 [5.53, 6.64]
Total SFAs	25.10 [24.39, 25.82]	24.74 [24.31, 25.17]	24.42 [23.27, 25.57]	24.31 [23.66, 24.96]
16:1n-7	1.83 [1.44, 2.23]	1.61 [1.46, 1.77]	4.51 [2.23, 5.78]	3.83 [3.27, 4.39]
18:1n-9	11.09 [9.94, 12.24]	11.24 [10.78, 11.70]	22.03 [19.63, 24.44]	19.68 [18.04, 21.33]
18:1n-7	2.74 [2.48, 3.00]	2.88 [2.82, 2.94]	4.23 [3.78, 4.68]	4.46 [4.38, 4.55]
20:1n-9	1.41 [1.21, 1.62]	1.28 [1.15, 1.42]	2.93 [2.38, 3.48]	2.58 [2.06, 3.10]
22:1n-11	0.71 [0.53, 0.88]	0.71 [0.54, 0.88]	1.95 [1.35, 2.54]	1.61 [0.76, 2.45]
Total MUFAs	19.08 [17.35, 20.80]	19.42 [18.86, 19.98]	37.84 [35.00, 40.68]	34.89 [33.97, 35.81]
18:2n-6	2.98 [2.09, 3.86]	1.91 [1.53, 2.29]	6.02 [4.47, 7.57]	3.50 [1.86, 5.14]
20:4n-6	2.55 [2.12, 2.98]	2.69 [2.42, 2.96]	1.43 [1.07, 1.79]	1.33 [1.16, 1.49]
Total n-6 PUFAs	7.23 [6.33, 8.14]	6.33 [5.52, 7.14]	9.17 [7.32, 11.03]	6.67 [4.54, 8.81]
18:3n-3	0.74 [0.53, 0.95]	0.69 [0.62, 0.76]	1.46 [1.02, 1.90]	1.46 [1.18, 1.73]
18:4n-3	0.52 [0.38, 0.65]	0.60 [0.45, 0.74]	1.44 [1.15, 1.73]	1.77 [1.34, 2.20]
20:5n-3	15.05 [14.35, 15.75]	14.31 [13.38, 15.23]	12.31 [10.06, 14.56]	12.69 [11.69, 13.69]
22:5n-3	1.80 [1.61, 1.98]	2.36 [1.96, 2.76]	1.23 [0.97, 1.50]	2.04 [1.60, 2.48]
22:6n-3	29.27 [27.15, 31.39]	30.17 [28.37, 31.97]	10.24 [8.04, 12.45]	14.44 [11.67, 17.21]
Total n-3 PUFAs	48.06 [46.37, 49.75]	48.89 [47.95, 49.83]	27.55 [23.39, 31.70]	33.46 [30.88, 36.05]
Total PUFAs	55.81 [54.01, 57.63]	55.85 [55.11, 56.57]	37.75 [34.03, 41.46]	40.80 [39.42, 42.18]
n-3/n-6	6.75 [5.88, 7.63]	7.88 [6.59, 9.18]	3.17 [2.31, 4.03]	5.90 [3.08, 8.72]

Table 4.6 Results of the analysis of variance (ANOVA) models for lipid and fatty acid analysis in mackerel muscle and liver tissues sampled near and away from a halibut farm. ***Note****:* Df: degrees of freedom, Sum Sq: Sum of squares, Mean Sq: Mean of squares, Significance level: $P < 0.05$.

	Mackerel muscle					Mackerel liver				
Total Lipids										
	Df	Sum Sq	Mean Sq	F value	P (> F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	4.511	4.511	3.252	0.080	1	0.002	0.002	1.231	0.281
Residuals	21	26.361	1.387			22	0.027	0.001		
14:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	1.160	1.161	0.686	0.418	1	0.016	0.016	0.335	0.570
Residuals	19	32.140	1.692			19	0.895	0.047		
16:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	7.450	7.449	2.216	0.153	1	40.260	40.260	4.930	0.039
Residuals	19	63.870	3.362			19	155.170	8.170		
18:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.494	0.494	0.510	0.484	1	4.511	4.511	3.252	0.087
Residuals	19	18.408	0.969			19	26.361	1.387		
Total SFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	26.250	26.247	8.057	0.011	1	72.750	72.750	5.096	0.036
Residuals	19	61.900	3.258			19	271.250	14.280		
16:1n-7										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.035	0.035	0.057	0.814	1	0.052	0.052	0.093	0.764
Residuals	19	11.740	0.618			19	10.535	0.555		

18:1n-9										
	Df	Sum Sq	Mean Sq	F value	P (> F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	118.500	118.550	3.361	0.083	1	19.900	19.920	0.500	0.488
Residuals	19	670.100	35.270			19	756.500	39.820		
18:1n-7										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	0.008	0.008	0.010	0.922	1	0.265	0.265	0.230	0.637
Residuals	19	15.370	0.809			19	21.862	1.151		
20:1n-9										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.508	1.508	0.957	0.340	1	2.432	2.433	4.056	0.058
Residuals	19	29.935	1.575			19	11.394	0.600		
22:1n-11										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.170	0.166	0.023	0.882	1	7.125	7.125	5.928	0.025
Residuals	19	138.680	7.299			19	22.835	1.202		
Total MUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	146.400	146.440	3.666	0.071	1	1.700	1.730	0.055	0.817
Residuals	19	759.000	39.950			19	601.700	31.670		
18:2n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	21.070	21.071	3.676	0.070	1	16.220	16.223	4.416	0.056
Residuals	19	108.910	5.732			19	74.350	3.913		
20:4n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.003	0.003	0.042	0.840	1	0.385	0.385	2.789	0.111
Residuals	19	1.399	0.074			19	2.624	0.138		
n-6 PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	21.030	21.034	3.627	0.072	1	30.140	30.137	4.075	0.058
Residuals	19	110.290	5.799			19	140.510	7.395		

18:3n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.086	0.086	0.236	0.632	1	0.663	0.663	2.819	0.110
Residuals	19	6.895	0.363			19	4.472	0.235		
18:4n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.906	1.906	8.441	0.009	1	0.015	0.015	1.024	0.324
Residuals	19	4.290	0.226			19	0.269	0.014		
20:5n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	10.690	10.685	7.988	0.011	1	1.328	1.328	1.275	0.273
Residuals	19	25.420	1.338			19	19.791	1.042		
22:5n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.006	0.006	0.121	0.732	1	1.940	1.941	1.014	0.327
Residuals	19	1.001	0.053			19	36.380	1.915		
22:6n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	47.700	47.690	1.634	0.217	1	4.310	4.306	0.875	0.361
Residuals	19	554.600	29.190			19	93.480	4.920		
Total n-3 PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	136.100	136.100	4.073	0.058	1	3.000	2.998	0.113	0.740
Residuals	19	634.700	33.400			19	503.000	26.476		
Total PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	48.700	48.690	1.352	0.259	1	52.000	51.990	0.875	0.361
Residuals	19	684.400	36.020			19	1129.000	59.400		
n-3/n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	50.120	50.120	5.011	0.037	1	87.170	87.170	9.474	0.006
Residuals	19	190.020	10.000			19	174.820	9.200		

Table 4.7 Results of the analysis of variance (ANOVA) models for lipid and fatty acid analysis in saithe musle and liver tissues sampled near and away from a fish farm. ***Note**:* Df: degrees of freedom, Sum Sq: Sum of squares, Mean Sq: Mean of squares. Significance level: $P < 0.05$.

	Saithe muscle					Saithe liver				
Total Lipids										
	Df	Sum Sq	Mean Sq	F value	P (> F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.000	0.000	0.012	0.918	1	0.000	0.000	0.014	0.909
Residuals	12	0.000	0.000			12	0.149	0.012		
14:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.413	0.413	8.853	0.012	1	0.9309	0.9309	5.285	0.040
Residuals	12	0.560	0.047			12	2.1136	0.1761		
16:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.726	1.726	8.391	0.013	1	1.984	1.984	2.68	0.128
Residuals	12	2.468	0.206			12	8.884	0.740		
18:0										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.917	1.917	23.550	0.000	1	0.206	0.206	0.312	0.587
Residuals	12	0.979	0.081			12	7.936	0.661		
Total SFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.472	0.472	1.154	0.304	1	0.041	0.041	0.040	0.845
Residuals	12	4.906	0.409			12	12.196	1.016		
16:1n-7										

	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.169	0.169	1.642	0.224	1	1.612	1.612	1.424	0.256
Residuals	12	1.238	0.103			12	13.580	1.132		
18:1n-9										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.077	0.077	0.086	0.774	1	19.320	19.317	3.888	0.072
Residuals	12	10.692	0.891			12	59.620	4.969		
18:1n-7										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.069	0.069	1.657	0.222	1	0.192	0.192	1.563	0.235
Residuals	12	0.502	0.042			12	1.475	0.123		
20:1n-9										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.062	0.062	1.774	0.208	1	0.429	0.429	1.284	0.279
Residuals	12	0.418	0.035			12	4.006	0.334		
22:1n-11										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.000	0.000	0.003	0.955	1	0.406	0.406	0.646	0.437
Residuals	12	0.421	0.035			12	7.549	0.629		
MUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.411	0.411	0.215	0.652	1	30.370	30.370	5.834	0.033
Residuals	12	23.011	1.918			12	62.470	5.206		
18:2n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	4.002	4.002	7.383	0.019	1	22.230	22.226	7.470	0.018
Residuals	12	6.504	0.542			12	35.700	2.975		
20:4n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.065	0.064	0.432	0.523	1	0.035	0.035	0.384	0.547
Residuals	12	1.791	0.149			12	1.095	0.091		

n-6 PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	2.849	2.849	3.316	0.094	1	21.850	21.850	4.671	0.052
Residuals	12	10.308	0.859			12	56.130	4.678		
18:3n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.009	0.009	0.321	0.581	1	0.000	0.000	0.000	1.000
Residuals	12	0.337	0.028			12	1.876	0.156		
18:4n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.022	0.022	0.945	0.350	1	0.381	0.381	2.435	0.145
Residuals	12	0.279	0.023			12	1.879	0.157		
20:5n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.931	1.931	2.462	0.143	1	0.500	0.502	0.141	0.713
Residuals	12	9.414	0.785			12	42.570	3.548		
22:5n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	1.109	1.109	9.775	0.009	1	2.284	2.284	15.070	0.002
Residuals	12	1.361	0.113			12	1.819	0.152		
22:6n-3										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	2.810	2.813	0.621	0.446	1	61.510	61.510	8.407	0.013
Residuals	12	54.370	4.531			12	87.800	7.320		
Total n-3 PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	2.399	2.399	1.095	0.316	1	122.6	122.57	8.765	0.0119
Residuals	12	26.286	2.191			12	167.8	13.98		
Total PUFAs										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	0.002	0.002	0.001	0.977	1	32.6	32.60	3.552	0.084

Residuals	12	26.755	2.230			12	110.2	9.18		
n-3/n-6										
	Df	Sum Sq	Mean Sq	F value	P (>F)	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	4.514	4.514	3.179	0.100	1	26.070	26.071	5.124	0.043
Residuals	12	17.041	1.420			12	61.060	5.088		

Table 4.8 Results of the nonparametric Mann-Whitney U models for lipid and fatty acid analysis in mackerel muscle and liver tissues samples near and away from a fish farm. ***Note:*** W: the test statistic, 95% confidence interval (CI), Significance level: $P < 0.05$.

	Mackerel muscle			Mackerel liver		
	W	**P-value**	**95% CI**	**W**	**P-value**	**95% CI**
Total lipids	95	0.067	-0.003, 0.082	97	0.285	-1.120, 6.870
14:0	42	0.378	-1.875, 0.524	64	0.557	-0.175, 0.265
16:0	45	0.512	-3.405, 0.780	26	0.043	-5.785, -0.015
18.0	42	0.387	-1.320, 0.730	33	0.132	-2.215, 0.120
Total SFAs	23	0.024	-4.165, -0.160	27	0.051	-7.360, 0.000
16:1n-7	58	0.863	-0.780, 0.760	59	0.809	-0.485, 0.660
18:1n-9	81	0.072	-0.925, 10.700	50	0.756	-8.790, 3.850
18:1n-7	45	0.512	-0.990, 0.920	59	0.805	-0.985, 1.290
20:1n-9	63	0.605	-0.645, 1.610	81	0.072	-0.065, 1.570
22:1n-11	47	0.605	-2.285, 2.335	79	0.099	-0.075, 2.375
Total MUFAs	87	0.024	0.885, 11.990	60	0.756	-4.730, 5.620
18:2n-6	56.5	0.944	-0.455, 5.575	78	0.113	-0.070, 4.55
20:4n-6	53	0.918	-0.240, 0.245	81	0.072	-0.045, 0.520
n-6 PUFAs	65.5	0.481	-0.645, 5.485	85	0.0357	0.060, 5.100
18:3n-3	44	0.468	-0.530, 0.765	72.5	0.231	-0.065, 0.900
18:4n-3	23	0.024	-1.130, -0.170	70	0.307	-0.065, 0.135
20:5n-3	20	0.0127	-2.405, -0.655	75	0.173	-0.315, 1.425
22:5n-3	59.5	0.778	-0.215, 0.260	70	0.314	-0.215, 2.060
22:6n-3	26	0.0430	-7.980, -0.330	39	0.282	-2.660, 0.960
n-3-PUFAs	21	0.0159	-11.065, -0.535	57	0.918	-3.125, 5.360
Total PUFAs	33	0.132	-9.340, 1.310	65	0.512	-2.860, 8.380
n-3/n-6	30	0.085	-7.165, 0.131	16	0.0048	-6.667, -1.110

Table 4.9 Results of the nonparametric Mann-Whitney U models for lipid and fatty acid analysis in saithe muscle and liver tissues samples near and away from a fish farm. ***Note:*** W: the test statistic, 95% confidence interval (CI), Significance level: $P < 0.05$.

	Saithe muscle			**Saithe liver**		
	W	**P-value**	**95% CI**	**W**	**P-value**	**95% CI**
Total lipids	9	0.0503	-0.003, 0.000	26	0.902	-0.132, 0.140
14:0	40.5	0.047	0.000, 1.000	33	0.318	-0.405, 1.170
16:0	33	0.318	-1.150, 1.260	19	0.535	-2.230, 2.390
18:0	11	0.097	-1.000, 0.315	22	0.805	-0.910, 0.795
Total SFAs	28	0.7104	-0.715, 1.500	27	0.805	-0.825, 1.320
16:1n-7	35	0.209	-0.150, 1.405	27	0.805	-1.470, 2.440
18:1n-9	31	0.456	-2.010, 7.705	31	0.456	-7.960, 3.745
18:1n-7	22	0.798	-0.495, 0.860	18	0.456	-1.355, 0.405
20:1n-9	36	0.165	-0.150, 1.050	26	0.902	-0.730, 1.205
22:1n-11	28.5	0.654	-0.415, 0.935	30	0.535	-0.800, 1.405
Total MUFAs	30	0.535	-2.645, 14.925	32	0.383	-15.145, 7.400
18:2n-6	48	0.001	0.365, 2.765	37	0.128	-0.835, 3.900
20:4n-6	21	0.701	-1.095, 0.395	31	0.456	-0.470, 0.830
n-6 PUFAs	38	0.097	-0.265, 3.455	36	0.165	-0.87, 4.59
18:3n-3	32	0.383	-0.210, 0.305	25	1.000	-0.680, 0.645
18:4n-3	23	0.902	-0.320, 0.360	15	0.259	-0.815, 0.345
20:5n-3	27	0.805	-1.025, 2.515	27	0.805	-2.455, 2.605
22:5n-3	6	0.017	-1.230, -0.080	3	0.004	-1.255, -0.170
22:6n-3	16	0.318	-17.400, 2.625	16	0.318	-8.455, 15.065
n-3 PUFA	16	0.318	-18.530, 1.595	17	0.383	-13.005, 13.285
Total PUFAs	20	0.62	-13.375, 2.805	19	0.535	-7.99, 13.90
n-3/n-6	7	0.026	-4.446, -0.270	15	0.259	-4.624, 2.133

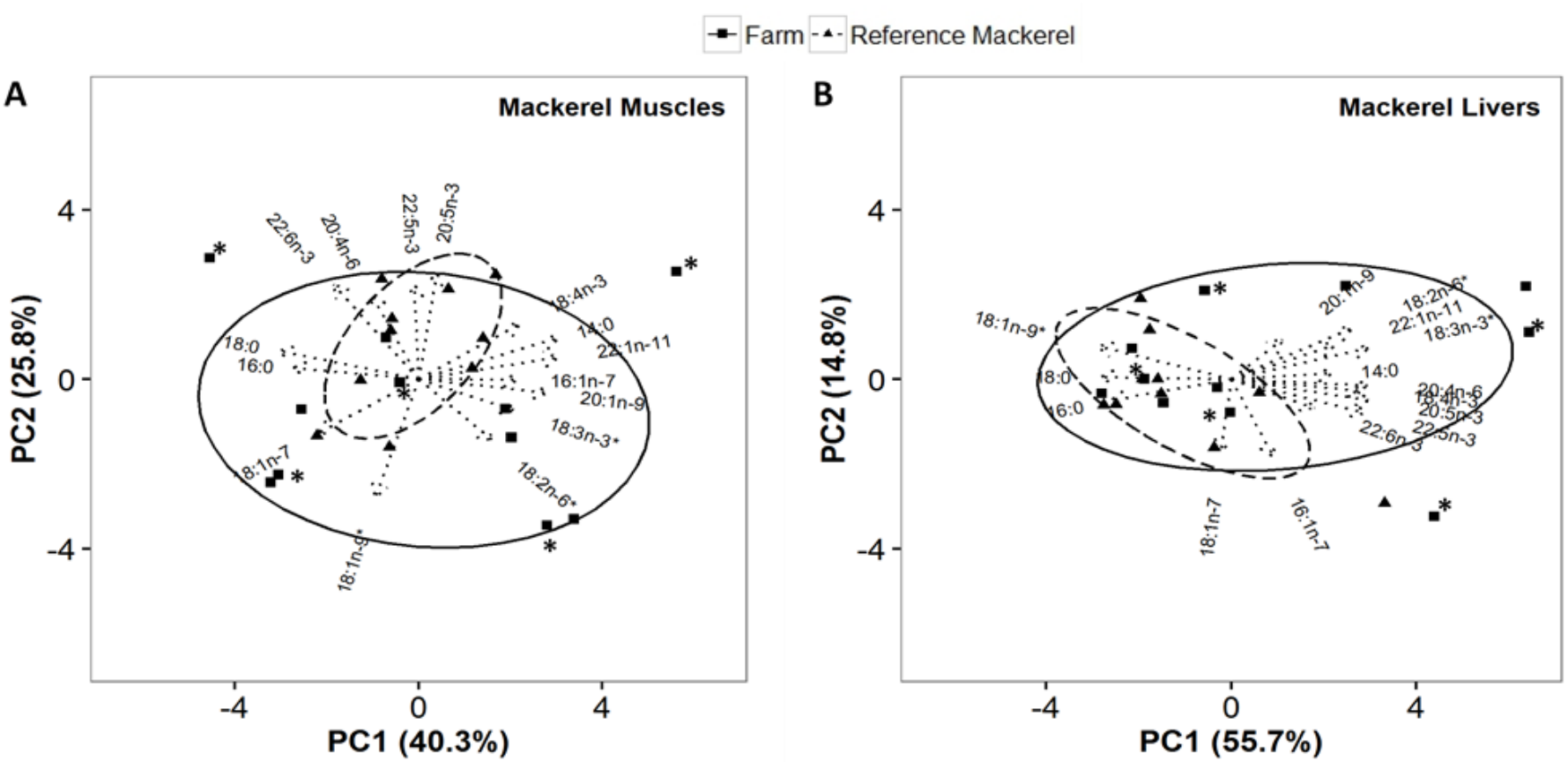

Figure 4.9 Biplots of the fatty acid composition of mackerel collected near (Farm) and away from a halibut farm (Reference Mackerel); A) PCA axes 1 and 2 for fillets and B) PCA axes 1 and 2 for livers. The ellipse shows the 95% variance for each group. ***Note:*** FAs (18:2n-6, 18:3n-3 and 18:1n-9) with an asterisk are potential FA biomarkers for a halibut farm influence. Fish found with waste feed in stomachs are denoted with an asterisk.

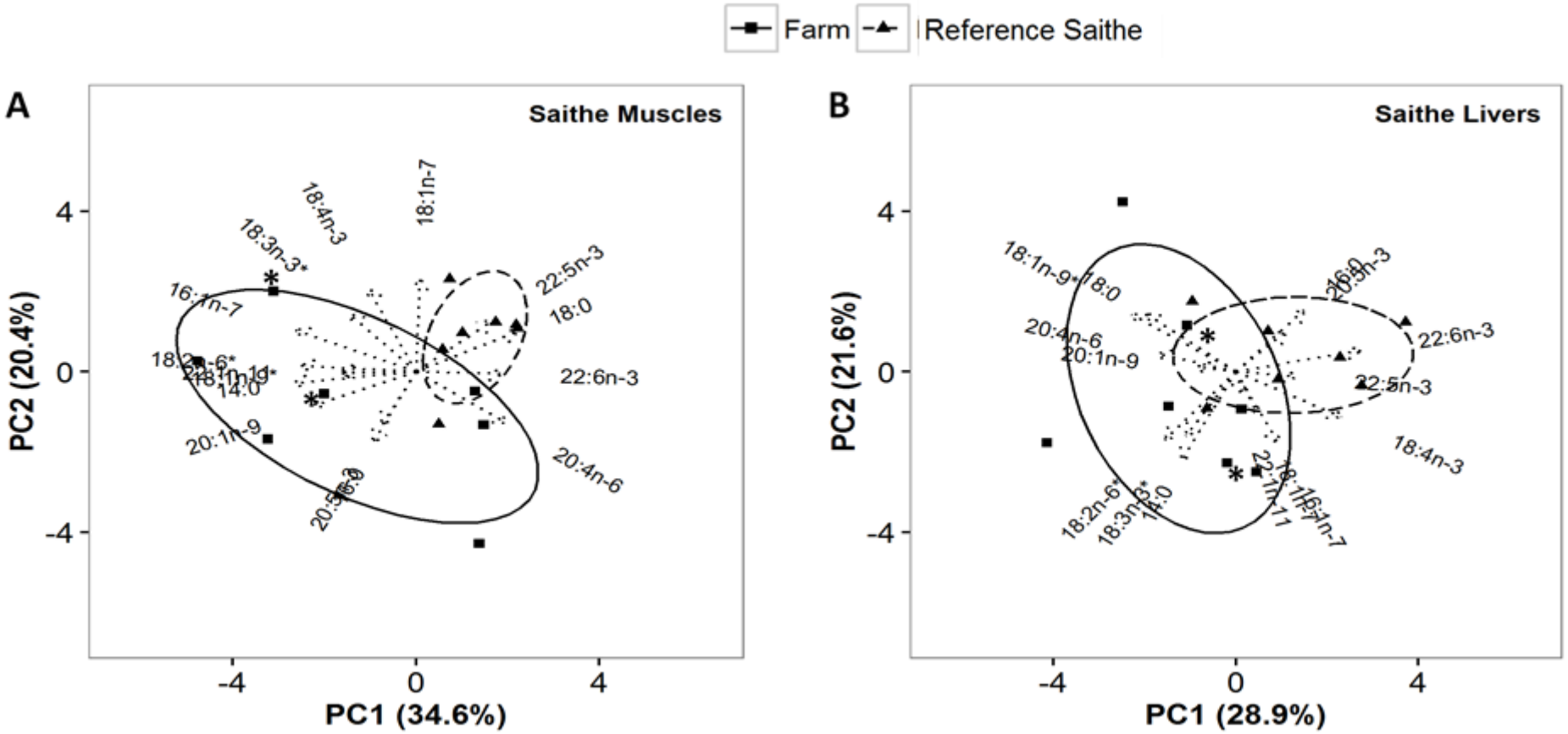

Figure 4.10 Biplots of the fatty acid composition of saithe collected near (Farm) and away from a halibut farm (Reference Saithe); A) PCA axes 1 and 2 for fillets and B) PCA axes 1 and 2 for livers. The ellipse shows the 95% variance for each group. ***Note:*** FAs (18:2n-6, 18:3n-3 and 18:1n-9) with an asterisk are potential biomarkers for a halibut farm influence. Fish found with waste feed in stomach are denoted with an asterisk

4.4 Discussion

It is evident from this study that a halibut farm increased the amount of food available (waste feed) to fish and other marine organisms around the cages. During the month of sampling (September 2013) at Loch Melfort, the farmed halibut were offered 19993 kg of artificial feed (see Table 3.1; Chapter 3). Assuming a maximum of 5% feed wastage (Gillibrand et al. 2002) the estimated amount of feed lost through cages in September 2013 was about 1000 kg. The results of this study indicated that both mackerel and saithe sampled in the vicinities of the halibut farm can consume waste feed which was associated with the FA modifications in both muscle and liver tissues. The study also showed that the FCI was improved for some mackerel sampled near the sea cages as compared to those sampled away from the sea cages. The HSI of some mackerel sampled near the sea cages was lower than for those sampled away from the sea cages. The FCI and HSI of saithe sampled near the sea cages was improved as compared to those fish sampled away from the sea cages. The overall impact of the fish farm was more evident in saithe than in mackerel.

4.4.1 Impacts of the halibut farm on mackerel

Mackerel visits the sea lochs during the summer to search for food. In open waters mackerel consumes mainly zooplankton whereas in coastal waters diet of mackerel appears to be dominated by fish (see Skaret et al. 2015 and references therein). Based on results from this study the diet of mackerel in the sea loch was dominated by clupeids (see Figure 4.2 and Appendix A). Possibly, clupeids also visit the sea lochs to feed on zooplankton and zooplankton enters the sea loch to feed on phytoplankton (e.g. Ross et al. 1994).

Presence of a halibut farm in Loch Melfort provided additional food for mackerel and other marine organisms. Mackerel consumed waste feed from the halibut farm which was evident in the stomach content analysis and was also captured on the underwater video recordings taken around the sea cages (see Figure 4.3 and Appendix A). Very few mackerel stomachs were found empty near the fish farm which indicates that food is not limiting. Clupeids were also noted on the underwater video recordings to feed either on plankton and/or organic matter (e.g. faeces) from the farm (see Appendix A).

The increase in nutrients from fish farms can potentially lead to increase in phytoplankton growth which can be a source of food for zooplankton and in turn for

higher trophic levels (Islam 2005; Sanchez-Jerez et al. 2011; Price et al. 2015). The flushing time of loch Melfort is nine days which indicates that the time of flushing is longer than the time for phytoplankton growth (~3-5 days) (see Chapter 3; Jones and Gowen 1985 cited in Mente et al. 2008; Olsen et al. 2008). Although, there is potential for phytoplankton growth and biomass accumulation within Loch Melfort the tidal cycles and fluxes in freshwater inputs in the loch are likely to reduce this potential (Gowen and Ezzi 1992 cited in Mente et al. 2008). Fish farming can increase nutrient levels but it is less likely to cause primary production in most Scottish lochs because of light limitation and circulation (Tett et al. 2011; Price et al. 2015).

The majority of mackerel sampled near the fish farm were longer and heavier than those caught away from the sea cages. One explanation for this is that the abundance of food resources around the sea cages are higher than those from a reference site resulting in differences in growth rate. Similarly, Skog et al. (2003) noted that saithe sampled near a Norwegian fish farm were significantly longer than those sampled from reference sites and was related to the presence of high energy waste feed. Another explanation is that when mackerel migrate they segregate by size in such a way that larger fish of a certain age reach spawning areas first and also leave for feeding grounds earlier than smaller fish (Lockwood 1988). Mackerel is expected to be in poorer condition when it arrives to the feeding grounds because they have used up the energy for migration and maturation of gonads and spawning. Therefore, larger fish near fish farms may have arrived earlier and some individuals may have stayed longer to benefit from the abundant food resources near the farm. It has been noted that majority of farm associated fish are of adult size (Dempster et al. 2002; Fernandez-Jover et al. 2007a) which appears to be the case for mackerel. Age analysis of fish was not conducted in this study. However, using length at age key for mackerel (see Appendix A) the approximate age for fish around the cages ranged from 1-6 years whereas those away from cages ranged from 0-2 years.

The parametric models indicated that the FCI, HSI and total lipids did not differ significantly between mackerel caught near the sea cages and their counterparts. The lack of differences in condition between fish near and away from sea cages can be because mackerel in both locations feed on high energy items. Anthony et al. (2000) noted that piscivorous fish can increase their energy intake through prey selection (e.g. high lipid fish) and by maximizing prey quantity. It is worth noting that the mackerel from the reference site is only about a kilometre away from the cages. Thus, some of the mackerel may have visited the farm and fed on prey available at the farm.

Although no significant differences were detected in condition indices of mackerel sampled near and away from the farm, correlation analysis revealed some patterns in the data. Some of the mackerel that were sampled near the fish farm were heavier, had higher total lipid content in the muscle tissues, had higher FCI and lower HSI. Total lipid content in muscle tissues was correlated with FCI in both groups of mackerel. Similar results were reported by Grégoire et al. (1994) in mackerel sampled in the Gulf of St. Lawrence, Canada. Hemre et al. (1997) noted a high correlation between mass and lipid content of mackerel reared in cages and fed a high energy salmon diet. Similarly, Wallace (1991) reported higher lipid content in heavier mackerel caught in the western English Channel. It was also noted that the heavier mackerel were mostly mature and contained high lipid levels that could be used for reproduction (Wallace 1991). Morse (1980) reported linear relationship between fecundity and mass for mackerel caught in the Middle Atlantic Bight. Thus, adult mackerel near the sea cages can potentially benefit from the food availability in terms of improved fecundity. Female mackerel with eggs have been caught near sea cages along the West Coast (Dr. Tom Wilding, SAMS, pers. comm., January 2017). Further research is needed to explore whether fish farming improves fecundity in fish around sea cages.

Heavier mackerel and also those sampled near the sea cages appear to have lower HSI than those sampled from a reference site. Similar, results were noted for horse mackerel sampled near two Meditteranean fish farms (Fernandez-Jover et al. 2007a). Fernandez-Jover et al. (2007a) noted that abnormal HSI values might be related to the presence of hormonally active compounds in the artificial feed that could activate hepatic enzymes which in turn would affect the liver weight (Sloof et al. 1983). Another explanation could be that in some species such as tuna individuals of low mass initially store fat in the liver and in heavier fish it is in the muscle (Clay 1988) which appears to be the case for mackerel in this study.

Vegetable oil replacement in diets for farmed halibut are not as high as those for farmed salmon (Alves Martins et al. 2011). Nevertheless, using FA biomarkers (18:2n-6, 18:3n-3 and 18:1n-9) to detect impacts of the halibut farm revealed that some individual mackerel sampled near the sea cages consumed the waste feed. This is consistent with the stomach content of mackerel sampled near sea cages where about a quarter of the individuals had consumed artificial pellets. The n-3/n-6 ratios in both muscle and liver tissues were significantly lower for mackerel sampled near the sea cages than their

counterparts and this result was robust whether the ANOVA or non-parametric tests were used.

The change in n-3/n-6 ratio is indicative of consumption of vegetable oil diet (e.g. Alves Martins et al. 2011). Hemre et al. (1997) reported that mackerel held in sea cages and fed high energy salmon diet had lower n-3/n-6 ratio after 8 months. Lower levels of n-3/n-6 ratios were reported for saithe (Skog et al. 2003) and horse mackerel (Fernandez-Jover et al. 2007a) sampled near sea cages in Norway and the Mediterranean Sea, respectively.

Scatter plots of selected FAs revealed that some mackerel sampled near the sea cages had elevated levels of 18:2n-6, 18:3n-3, lower levels of n-3/n-6, and an overall better FCI and lower HSI than mackerel sampled away from the farm. The statistical analysis of individual FAs did not detect differences in most FAs. This is most likely because of the high variability in the data which was also noted in the PCA. Although the PCA analysis did not detect clear separation in both muscle and liver tissues of mackerel sampled near and away from the cages it indicated that mackerel near the sea cages had high variation in the FA profiles. Some of the mackerel sampled near the sea cages had elevated levels of 18:2n-6 and also waste feed was found in their stomachs. However, not all fish that had elevated levels of 18:2n-6 had consumed artificial feed. This indicates that mackerel spend varying times around the sea cages. Although the stomach content analysis revealed that the food items of mackerel are mainly fish and to a lesser extent waste feed the stomach content analysis only reveals the most recent meal consumed by the fish whereas FA reflect long term diet (see Chapter 2). Mackerel around the sea cages possibly have a wide choice of prey or their prey are feeding on different items. It is also worth noting that mackerel most likely visit other non-halibut fish farms that are within few kilometres of the halibut farm.

Differences in FA profiles may arise from the differences in age. Mackerel sampled around the cages are of different ages with majority being of adult ages whereas those from the reference site are of similar young age. Other factors such as sex and reproductive stage can affect the variability of FAs (Halver 1972). Other studies have also reported variability in FA profiles of fish sampled near sea cages (e.g. Skog et al. 2003; Fernandez-Jover et al. 2007a).

Lipids are stored in different parts of the body depending on the species (Jobling 2001). In mackerel, lipids are deposited throughout the body including muscle and liver tissues (Ackman and Eaton 1971; Ackman and Zhou 1994). Both muscle and liver tissues

were influenced by the dietary composition of mackerel and can be used to detect waste feed consumption.

4.4.2 Impacts of the halibut farm on saithe

Saithe are often observed in the vicinities of cage farms in Norway (Bjordal and Skar 1992; Skog et al. 2003; Dempster et al. 2009, 2011). Carss (1990) reported juvenile saithe as the most abundant species around sea cages in Loch Melfort, Scotland. In the present study, I did not find a high number of saithe near the cages which may be caused by the different sampling methods used in both studies; beach-seine netting in Carss (1990) and baited rod and line in the present study.

Saithe caught at reference site had high proportion of invertebrates in their guts and lower proportion of juvenile fish. No fish pellets were noted in their stomachs. Fish near the cages had mainly juvenile fish and a small proportion of fish pellets. A number of studies have found pellets in saithe near cages as compared to saithe caught away from cages (e.g. Carss 1990; Skog et al. 2003). Carss (1990) and Mente et al. (2008) reported that saithe caught near cages in few sea lochs were the only wild fish species to have eaten pelleted food. However, as revealed from this study and the next (Chapter 5) mackerel and whiting also consumed artificial pellets in addition to flatfish (see Appendix A). Similar to mackerel the opportunistic feeding behaviour of saithe (Tyrrell et al. 2007) allows the exploitation of various food resources near the fish farms including waste pellets.

Saithe sampled near the sea cages were of smaller size than those sampled at a reference site. Results of this study are in contrast with results reported by Skog et al. (2003) and Carss (1990) where saithe sampled near sea cages were longer as compared to those of reference sites. One explanation for the difference in size could be age related differences. Based on the length at age key (see Appendix A) both groups of saithe were of 0-age. It is worth noting that the halibut farm was located in a small bay where there were plenty of shelters for young fish whereas less shelters were available at the reference site.

Despite the smaller size of saithe sampled near the sea cages their FCI and HSI were higher than those of saithe sampled from a reference site. This can be explained by the availability of food including the high energy content waste feed around the cages. Similar reports have been reported for saithe sampled near fish farms in Norway (Skog

et al. 2003; Dempster et al. 2011). Another possible explanation for differences in FCI is possible differences in activity between saithe sampled near and away from sea cages. Availability of food resources decreases activity which can induce deeper body and therefore improved FCI (Johansson and Andersson 2009). Liver is the main organ of lipid accumulation in gadoids (Lambert and Dutil 1997) and high HSI has been linked to greater reproductive output in gadoid species (Marshall et al. 1999). As the saithe sampled near the cages are young fish the availability of favourable environmental conditions such as presence of high quality food or lipid content can lead to faster growth and earlier sexual maturation (Taranger et al. 2010). In gadoids, the age of sexual maturity is possibly dependent on the stored lipids (Eliassen and Vahl 1982).

The total lipid content in both muscle and liver tissues did not significantly differ between saithe sampled near and away from sea cages. However, the scatter plots revealed that the lipid content in muscle tissues was higher for saithe sampled near the sea cages as compared to their counterparts. The lipid level in the muscle decreased with length in both groups but increased in the liver tissues. Saithe near the sea cages may be exposed to plenty of high energy food that allows faster growth and lipid accumulation in the livers. Otterå et al. (2009) noted that when the diet of saithe was switched from low energy diet (cod diet; 18% lipid content) to high energy diet (salmon diet; 31-33% lipid content) the HSI of the fish increased. The researchers added that saithe stores excess energy in the liver and that even few weeks of feeding on waste feed can induce changes in composition (Otterå et al. 2009).

The consumption of waste feed by saithe near sea cages was also noted in the changes of the FA profiles. Significant differences were noted in 18:2n-6 levels of muscle and liver tissues in saithe sampled near and away from sea cages. Differences in n-3/n-6 levels for both groups of saithe were only significant for the liver tissues. Similar results were reported for saithe sampled near sea cages in Norway (Skog et al. 2003). Otterå et al. (2009) also reported that the levels of 18:2n-6 increased and n-3/n-6 decreased when saithe consumed a lipid rich salmon diet as compared to saithe that fed the lean cod diet. Scatter plots indicated that saithe sampled near the sea cages had an overall higher levels of 18:2n-6, 18:3n-3, 18:1n-9 and increased levels of FCI and HSI.

The PCA showed stronger patterns of separation between FA profiles of saithe caught near and away from cages than the statistical analysis of individual FAs. Not all fish that were found with elevated levels of 18:2n-6 near the sea cages had waste feed in the stomach. Similar to the results from the PCA of mackerel, PCA showed high

variability in FAs of saithe near farms which suggests that individuals spend different times near the cages. Similar patterns of high FA variability were also noted for saithe sampled near sea cages in Norway (Skog et al. 2003). Diversity in food items near the cages can be a reason for the variability in FA profiles of saithe. It is also worth noting that changes in the diet of the farmed fish lead to waste feed that is of different composition.

PCAs for both muscle and liver tissues showed similar patterns of separation between saithe sampled near and away from sea cages. However, the difference in the liver and muscle tissues is the predominance of some FAs and not others. For example, liver tissues have higher levels of MUFAs and lower levels of PUFAs as compared to muscle tissues in both groups of saithe. However, increase in biomarkers such as 18:2n-6 and changes in n-3/n-6 levels was noted in both tissues. Both muscle and liver tissues can be used to detect waste feed consumption by saithe.

4.4.3 Comparison between mackerel and saithe

Results of this study indicated that both mackerel and saithe sampled near a halibut farm can benefit from the abundance of food near the sea cages. Both species were noted to forage on commercial feed which was associated with changes in FA profiles in liver and muscle tissues. The impacts of the halibut farm appear to be stronger on saithe than on mackerel. One explanation for this is related to physiological and behavioural differences between the species. Mackerel is a pelagic fish that is most likely to take advantage of the waste pellets from the fish farms and other prey around the sea cages. On the other hand saithe is a benthopelagic species that feeds on the waste pellets but can potentially also consume more of the mixture of broken pellets and faeces under the sea cages. Mackerel lack swimbladder and need to continuously swim (Juell et al. 1998). This indicates that some mackerel continuously swim around the sea cages or swim between different fish farms within the loch or outside the loch.

Mackerel is a migratory species that visits the sea lochs during the summer (3-4 months) to search for food (Lockwood 1988). The presence of fish farms could affect migration patterns of the species with delayed offshore migrations. The halibut farm impacts mackerel of different ages whereas the impacts on saithe are mainly on young fish.

Generally, saithe spend 2-4 years in coastal waters and then migrate offshore to spawn (see Armannsson et al. 2007). Saithe also perform seasonal migrations to deeper waters during winter followed by return to shallower waters during summer (see Armannsson et al. 2007). Little is known about the saithe population in Loch Melfort but it is expected to show similar behaviour to other gadoids in other lochs. Hawkins et al. (1985) reported that cod settle in shallow parts of Loch Torridon during their first year of life and could remain in the loch until they are between 2 and 4 years old before the adult individuals leave to join the offshore population. Diurnal and seasonal activity has also been reported for gadoids including saithe (Hawkins et al. 1985; Nickell and Sayer 1998). Hawkins et al. (1985) noted that growth and condition for cod during winter is related to low food availability rather than the decrease in growth at low temperatures. Thus, presence of fish farming can potential supply waste feed during winter months which would benefit local juvenile gadoid populations. Additionally, sea lochs are used as nurseries for young gadoids (Ware 2009) where fish farms are located. As food is abundant around the sea cages young gadoids could stay longer in the lochs which would affect the migration patterns between coastal and offshore waters. Otterå and Skilbrei (2014) reported that migration patterns of saithe were altered by the presence of coastal fish farms in a Norwegian fjord.

Although mackerel and saithe show different behaviours, results of this study indicate that both mackerel and saithe spend sufficient time around the sea cages for physiological changes to occur. In Norway, a number of studies reported that wild saithe reside near the coastal fish farms for several months which is sufficient time to cause physiological changes when saithe feed on waste feed (see Bjordal and Skar 1992; Bjordal and Johnstone 1993; Skog et al. 2003; Uglem et al. 2009; Dempster et al. 2009, 2011; Fernandez-Jover et al. 2011a). Fernandez-Jover et al. (2007a) reported that horse mackerel is a resident around sea cages in the Mediterranean Sea for a maximum of 3-4 months which was sufficient time to detect changes in FA profiles.

4.4.4 Using fatty acids as biomarkers

The use of FA as biomarkers to detect the impacts of a halibut farm in both mackerel and saithe was useful. Methods such as stomach content analysis only give information on the most recently consumed meal whereas biochemical methods such as fatty acids and stable isotope analysis can give information on the long term diet of a fish

(Peterson and Fry 1987; Iverson et al. 2004; Budge et al. 2006; Boecklen et al. 2011). Stable isotope analysis estimates the trophic level of a predator but cannot determine the dietary composition (Boecklen et al. 2011). Olsen et al. (2015) used both FA determination and stable nitrogen analyses in a laboratory study to detect changes in FA profiles when cod were fed different diets. The authors noted that although both methods could detect dietary shifts fatty acid analysis is a better tracer particularly for specific lipids of terrestrial origin. Compound specific isotope analysis (CSIA) has been used by a number of researchers to reveal the origin of individual biomarkers in the diet of an animal (see Budge et al. 2016 and references therein). This should be considered in future work. Other less costly and time-consuming tools that have been used to detect the impacts of fish farming on wild fish populations is using nuclear magnetic resonance (Maruhenda Egea et al. 2015). The nuclear magnetic resonance spectroscopy can detect small molecules (e.g. metabolites such as glucose, amino acids) that could differentiate fish of different origins (see Maruhenda Egea et al. 2015).

Using muscle and liver tissues in both species was useful in detecting the influence of the farm. Muscle tissues can reflect changes in the diet of wild fish that have consumed commercial feed within about a month and similarly noted for liver (e.g. Gonzalez-Silvera et al. 2016). However, Gonzalez-Silvera et al. (2016) pointed out that the liver tissues are not only storage organ for lipid but also have metabolic activity which makes these tissues less suitable for tracking commercial feed in wild fish. Other tissues such as the brain are more conservative and can reflect long term dietary changes (~ 2 months for dietary change to be detected) (Gonzalez-Silvera et al. 2016). Gonzales-Silvera et al. (2016) suggested the use of muscle tissues in conjunction with brain tissue as more suitable tools to track waste feed in wild fish.

Based on the availability of resources, I have used mainly stomach content analysis and fatty acid analysis to detect the impacts of a halibut farm on mackerel and saithe. Both methods were useful in detecting waste feed consumption by both fishes. As every methodology has advantages and disadvantages a combination of methods would be a better approach in understanding the impacts of fish farming on wild fish populations.

4.4.5 Limitations of the study

The results of this study should be interpreted with caution as there are a number of limitations. The method of capture is by using hook and line which is size selective

method and more biased towards fish that are feeding. Some smaller or bigger fish may be underrepresented. Therefore, the results of this study does not represent all fish around sea cages but those caught using only this method of sampling. Other fishing methods such as gillnets or baited traps amongst others or a combination of methods should be explored.

The study only compares one fish farm with one reference site for each species. As there are no replication at the level of site the fish are pseudoreplicated which limits the capability of the study to generalise the results across all fish farms on the West Coast. Additionally, the selected farm is a halibut farm which is not a common fish farming activity along the West Coast. However, the farm is a fish farm and particulate organic matter is released which has a certain impact on the environment. The farm also consists of sea cages which act as fish aggregating devices. Therefore, in these terms it is similar to salmon farming which is the predominant fish farming activity on the West Coast (see Chapter 1).

The sampling size for both species is small which may have limited the detection of any statistically significant differences between fish sampled near and away from sea cages. The high variability in the data which was also reflected in wide confidence intervals for some variables of interest further limited the detection of significant differences.

The assumptions (e.g. normality, lack of homogeneity of variances) of the parametric models for some variables (e.g. FAs) were moderately violated. Some values appeared to be further apart from the rest of the data; however as mentioned in the results section these were not excluded from the data. These observations indicated that only a small proportion of the population sampled near the sea cages is impacted by the farm. This was also noted in the scatter plots and the PCA biplots.

In general, violations of assumptions in the ANOVA models would increase the probability of making type II error or the acceptance of a false null hypothesis. As noted in Chapter 2, transformations of the response variable could improve some of these violations. However, following some trials using logarithmic and square root transformations the assumptions were still not met. Thus, non-parametric tests (free distribution) were used for some of the variables. Overall the results of the non-parametric tests were similar to those of the parametric tests. It is important to note when the difference between two groups is large enough or there is no difference at all any statistical model should show this. The use of nonparametric tests are overall less

conservative with increased probability of making type I error or the rejection of a true null hypothesis. More advanced models such as generalised least square models were not used as no additional information would be obtained. Following the evaluation of the data using various techniques the parametric models used were the most appropriate as they reflect the ecological reality around the sea cages. This is also confirmed by using other statistical methods such as the scatter plots and the use of multivariate techniques.

The use of both univariate and multivariate approach was useful with PCA providing an overall better indication of differences in terms of FAs between farm and reference fish.

4.5 Conclusions

Using a combination of empirical methods indicated that both mackerel and saithe consume waste feed lost through the cages of a halibut farm. The impacts of the farm on diet, condition and FA profiles appeared to be stronger on saithe than on mackerel.

Results of this preliminary study are not conclusive as the study was conducted on a small sample of fish, one farm and one reference site for each species. The next study was extended to take into account sampling size, a second farm and 2-3 reference sites for each species of interest.

CHAPTER 5
USING FATTY ACID BIOMARKERS TO CONTRAST AND DISTINGUISH PHYSIOLOGICAL EFFECTS ON MACKEREL AND WHITING CAUGHT NEAR TWO FISH FARMS

5.1 Introduction

The response of species to anthropogenic disturbance can be beneficial for some (e.g. urbanised pests) and detrimental for others depending on the behavioural responses, life history, size of the species, and ability to adapt to the new environment (Toumainen and Candolin 2011). As results in Chapter 4 indicated that mackerel and saithe sampled near the halibut farm are affected in different ways which can be because of the ecological (migratory/residential behaviour, pelagic/benthic/benthopelagic feeding habits, adult/juvenile stage), and physiological differences. Similarly, it is expected that response of mackerel to fish farms would be different than those for gadoid species such as whiting.

To address the impacts that fish farming can have on wild marine fish communities it is important to understand how fish with different ecological roles are affected by these activities. Knowledge on how wild fish are affected can guide the site selection of fish farms, management of fish farming activities and wild fish stocks, and conservation of wild fish.

The aim of this study was to confirm the results of the previous study by evaluating the impacts of the same halibut farm on diet, condition and total lipid and FA profiles of mackerel and whiting sampled near the sea cages. LDA was used as a multivariate technique to distinguish the FA profiles of mackerel and whiting caught near sea cages and at reference sites. The study was also extended to a second farm. The second farm was a salmon farm which is the main fish farming activity along the West coast of Scotland (see Chapter 1).

5.2 Methods

Sampling methodologies and details on farm and control sites can be found in Chapter 3. Mackerel and whiting were sampled near a halibut and a salmon farm (Melfort and Leven farms) on the West Coast of Scotland (Figure 5.1A,B). Mackerel away from

cages were sampled at three reference sites located at least 2 km away from fish farms (Figure 5.1A). Whiting away from cages were sampled at three reference sites of more than 25 km away from fish farms (Figure 5.1B). A total of 308 mackerel and 190 whiting were sampled from all sites. As mentioned in Chapter 3, resources limited the choice and number of farms to be studied.

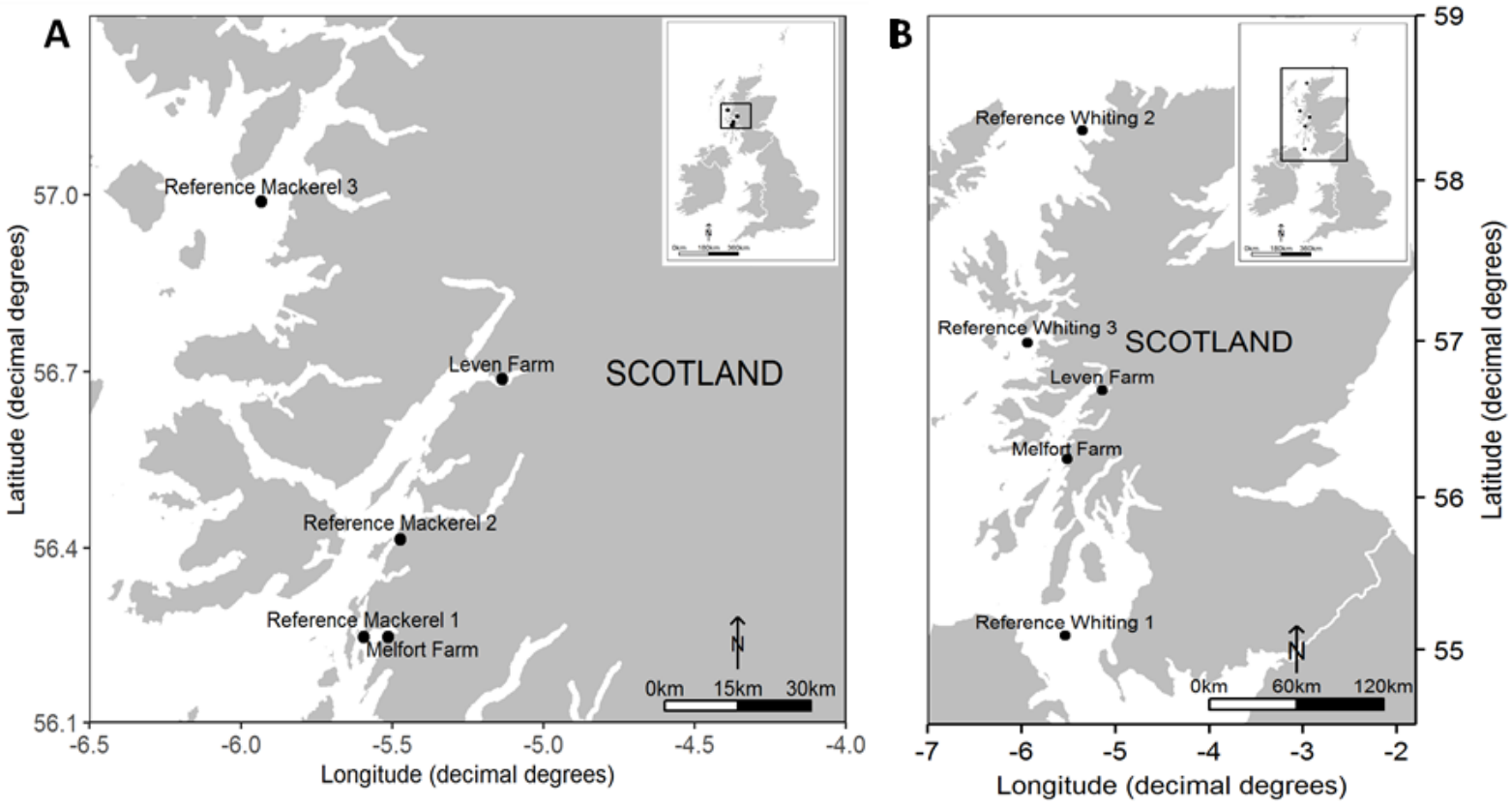

Figure 5.1 Sampling locations for mackerel A) and whiting B) near two fish farms (Melfort Farm and Leven Farm) and reference sites on the West Coast of Scotland for 2014.

5.2.1 Statistical analysis

All univariate and multivariate analysis were conducted using the statistical software R (R Development Core Team 2016) run in RStudio (version 1.0.136, RStudio Team 2016).

Prior to applying any statistical models to the data graphical exploratory tools (boxplots and Q-Q plots) were used as suggested by Zuur et al. (2010) (for more detail see Chapter 4). As in Chapter 4, scatter plots were used to explore the relationships between different variables. Frequency of occurrence of each group of items was plotted for both mackerel and whiting (see Chapter 4).

The experimental design was a nested one which consisted of one factor which is location with two levels (farm and control). For each level two or three replicates were used and fish were nested within each. For mackerel two farms and three references sites were used whereas for whiting two farms and two reference sites were used for the models. As mentioned in Chapter 3, the whiting for the third reference site were much bigger than all other reference sites and therefore were not used in the models. Summary statistics for the third reference site for whiting can be found in Appendix C. From a practical point of view it was not possible to select sites at random. The sampling protocol was dependent on the resources and the access to the different sites. Additionally, although the two farms cultured different fish species and therefore different diets were used, both farms release waste feed which has the potential to impact the wild fish around the fish farms. Both farms are also considered fish aggregating devices that can have an impact on wild fish (see Chapter 1). Therefore, for the statistical modelling both farms were assumed to be similar.

Linear mixed effects models are useful for incorporating inter-farm variation as a 'random' effect in which we allow for variation between farms without being concerned about the special features of any particular farm (see Chapter 2). Linear mixed effect models with site as random effect were used to evaluate whether there were differences in length, mass, FCI, HSI, lipid content and selected individual FAs of mackerel and whiting sampled near and away from sea cages. To assure that differences in FCI and HSI between fish sampled near and away from sea cages is not size related, length was taken as a covariate and dropped if found not significant.

Models were built using packages lme4 (Bates et al. 2015) with lmerTest (Kuznetsova et al. 2016) to approximate p-values, and using maximum likelihood estimation.

Model assumptions (normal distribution, homogeneity of variance, and linearity) were evaluated by visually inspecting the residuals and fitted values (Appendix C).

Linear discriminant analysis (LDA) is a multivariate technique that calculates the combination of FAs that produce the maximum multivariate distance among groups by creating uncorrelated linear equations of the original FAs (Budge et al. 2006). The main assumptions for LDA include that observations are independent, the covariance matrices are homogeneous and the data are multivariate normal (Budge et al. 2006). Budge et al. (2006) notes that these assumptions are rarely met with FA data and one should be aware of the limitations and potential effects on the interpretation of the results.

In this study, LDA was used to distinguish between fish sampled near the two fish farms and fish from control sites. For mackerel, LDA was used to distinguish among fish with two fish farms and three reference sites. For whiting it distinguished among fish sampled near the two fish farms and fish from two reference sites. For the analysis the same 15 selected FAs as in Chapter 4 were used for both species to distinguish between the different fish groups. LDA can also be used to classify new samples into groups based on the FA composition (Hair et al. 2006). The LDA was performed using the package MASS (Venables and Ripley 2002) with function lda. Packages ggplot2 (Wickham 2009) and cowplot (Wilke 2015) were used to plot the data.

5.3 Results

5.3.1 Observations (anecdotal accounts)

All details regarding species sampled using hook and line or observed with the underwater video camera in 2014 can be found in Appendix A. Seabirds were noted near the sea cages in Loch Melfort as in the previous year 2013. Two seals were noted throughout the fieldwork in Loch Melfort and one seal in Loch Leven. In Loch Melfort, it was overall relatively faster to catch fish near the sea cages as compared to Loch Leven. Two porpoises were noted at about 100 meters from the sea cages at Loch Melfort during one visit in 2014. Overall, more marine organisms were noted near the halibut farm than the salmon farm. It is worth noting that based on the cultured species requirements the halibut farm was located in a more sheltered area than the salmon farm. Jellyfish were

also noted at the start of fieldwork in both lochs with relatively more noted in Loch Melfort.

It is also worth noting that at both farms it was mentioned by farm staff that young juvenile gadoids enter the sea cages and could stay there until harvesting of the cultured species. This needs to be considered in any future studies related to fish farming impacts and wild fish communities.

5.3.2 Data patterns

Scatter plots were used to visualise the relationship between different variables (length, mass, FCI, HSI, and total lipids) for mackerel (Figure 5.2) and whiting (Figure 5.4) sampled near and away from sea cages. Scatter plots were also used to evaluate the relationship between condition and selected FAs for mackerel (Figure 5.3) and saithe (Figure 5.5).

5.3.2.1 Mackerel

Length and mass were positively correlated ($r = 0.96$, $p < 0.000$) for mackerel sampled near and away from sea cages (Figure 5.2). FCI was found positively correlated with FAs 18:2n-6 ($r = 0.32$, $p < 0.001$), 18:3n-3 ($r = 0.25$, $p < 0.01$), and 18:1n-9 ($r = 0.38$, $p < 0.000$) (Figure 5.3). The FA 18:2n-6 was positively correlated with 18:3n-3 and 18:1n-9 and negatively correlated with n-3/n-6 (Figure 5.3).

Overall mackerel sampled near the halibut and salmon farm were heavier and longer than mackerel sampled at reference sites (Figure 5.2). Few of the mackerel sampled near the halibut and salmon farm appeared to be heavier, longer and to have higher lipid contents than mackerel from reference sites (Figure 5.2). Few of the mackerel sampled at both farms had better FCI and higher levels of the FAs 18:2n-6 and 18:3n-3 and lower levels of n-3/n-6 ratios (Figure 5.3).

5.3.2.2 Whiting

Strong linear correlation was found for length and weight for whiting sampled near and away from sea cages (Figure 5.4). The HSI was positively correlated with length ($r = 0.74$, $p < 0.000$) (Figure 5.4). The FAs 18:2n-6, 18:3n-3 and 18:1n-9 were positively correlated with FCI and HSI (Figure 5.5). The FA 18:2n-6 was positively correlated with 18:3n-3 and 18:1n-9 and negatively correlated with the n-3/n-6 ratio (Figure 5.5).

Overall, some of the whiting near the salmon farm were of higher length and mass and had better HSI than whiting sampled from other sites (Figure 5.4). The whiting sampled near the halibut and the salmon farm had elevated levels of the FAs 18:2n-6, 18:3n-3 and 18:1n-9 and lower n-3/n-6 ratios. The whiting sampled near the salmon farm appeared to have higher HSI and elevated levels of the FA 18:2n-6 than those sampled away from the farm (Figure 5.5).

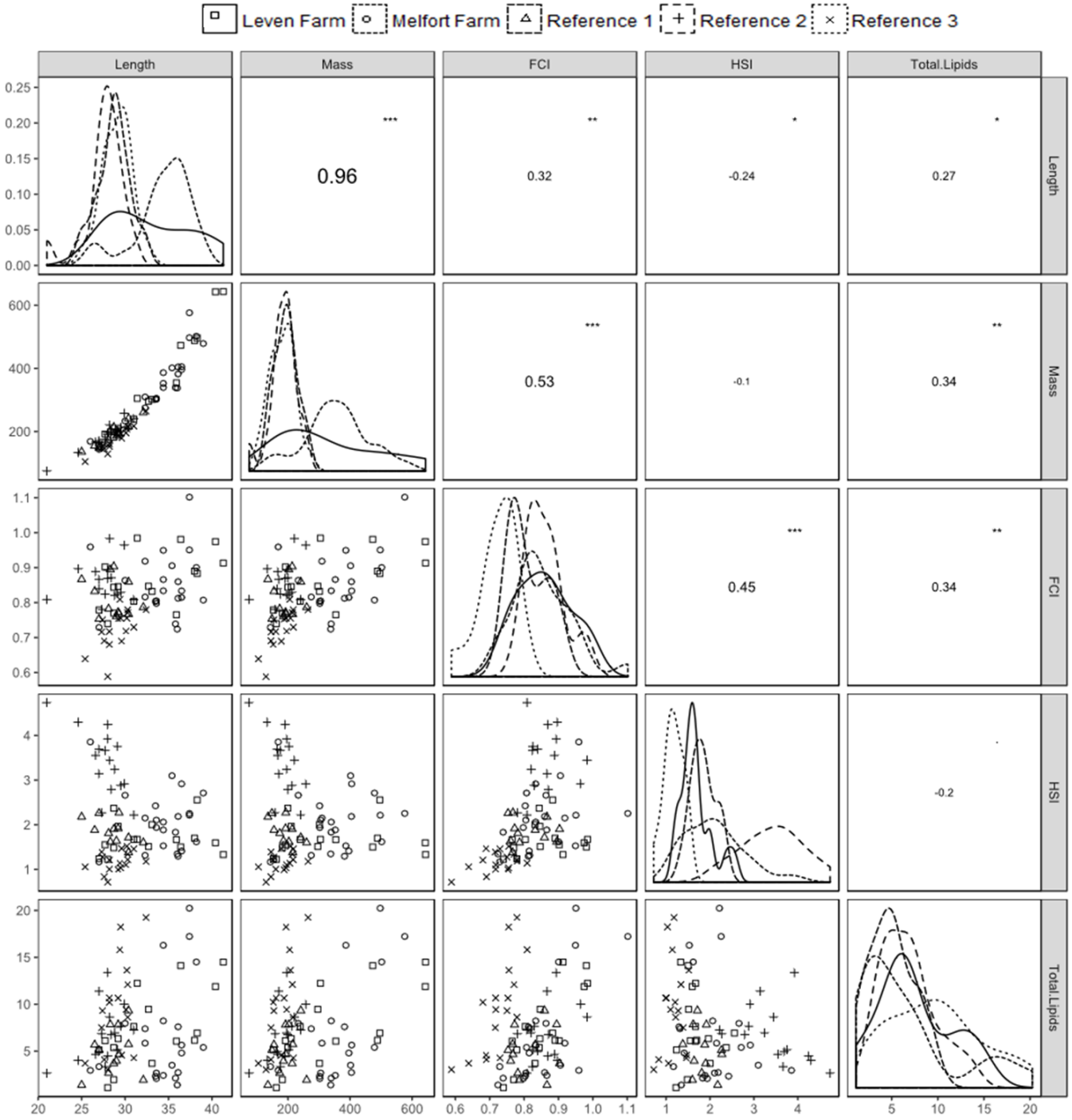

Figure 5.2 Scatter plots of length (cm), mass (g), FCI, HSI, and total lipid contents (%) in muscle of mackerel sampled near a salmon farm (Leven Farm), a halibut farm (Melfort Farm) and three reference sites (Reference 1, 2 and 3). Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

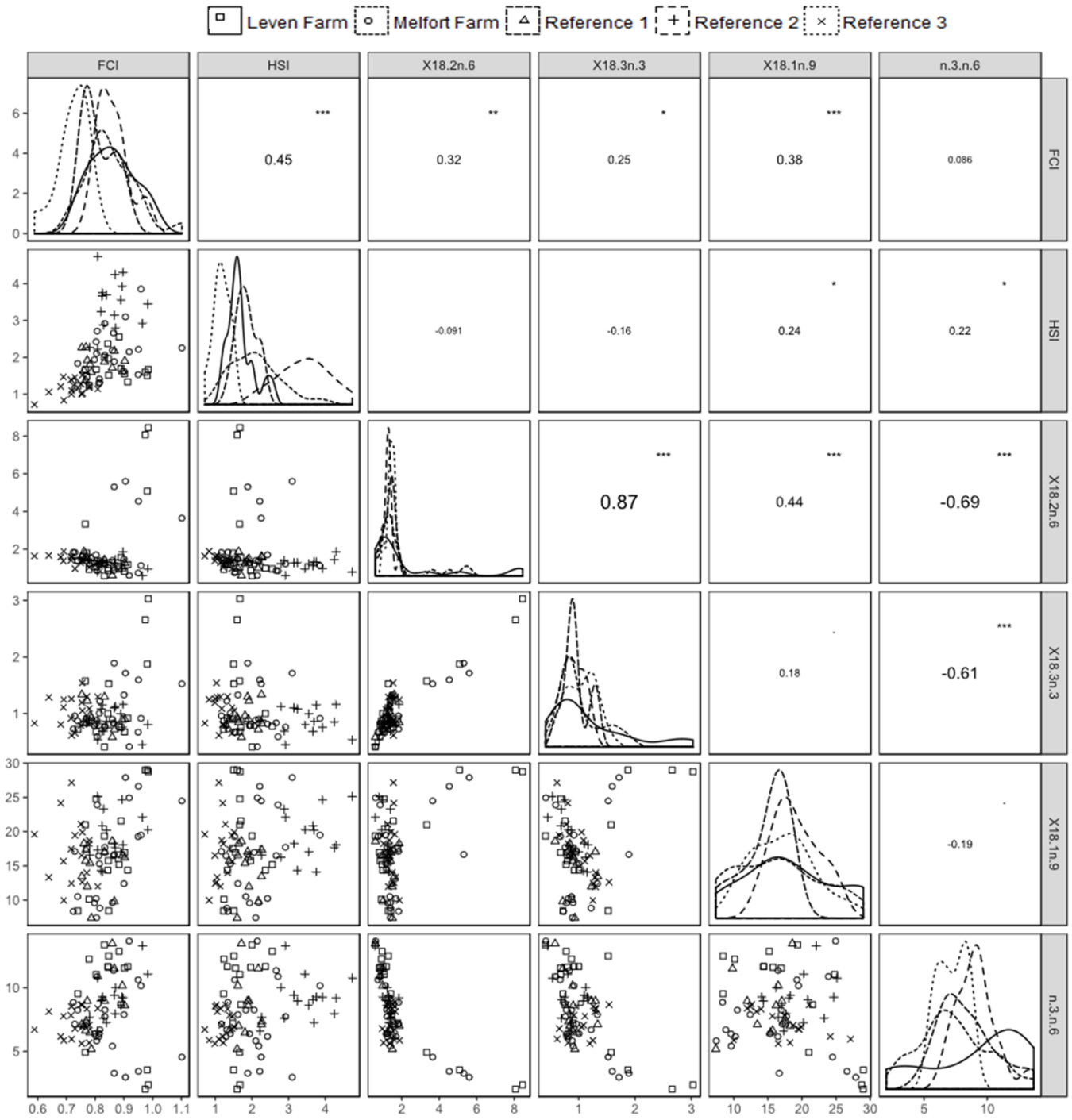

Figure 5.3 Scatter plots of FCI, HSI, and selected FAs (18:2n-6, 18:3n-3, 18:1n-9) and n-3/n-6 ratio in the muscle of mackerel caught near a salmon (Leven Farm) and a halibut farm (Melfort Farm) and reference sites (Reference 1, 2 and 3). Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient corresponds to the significance level.

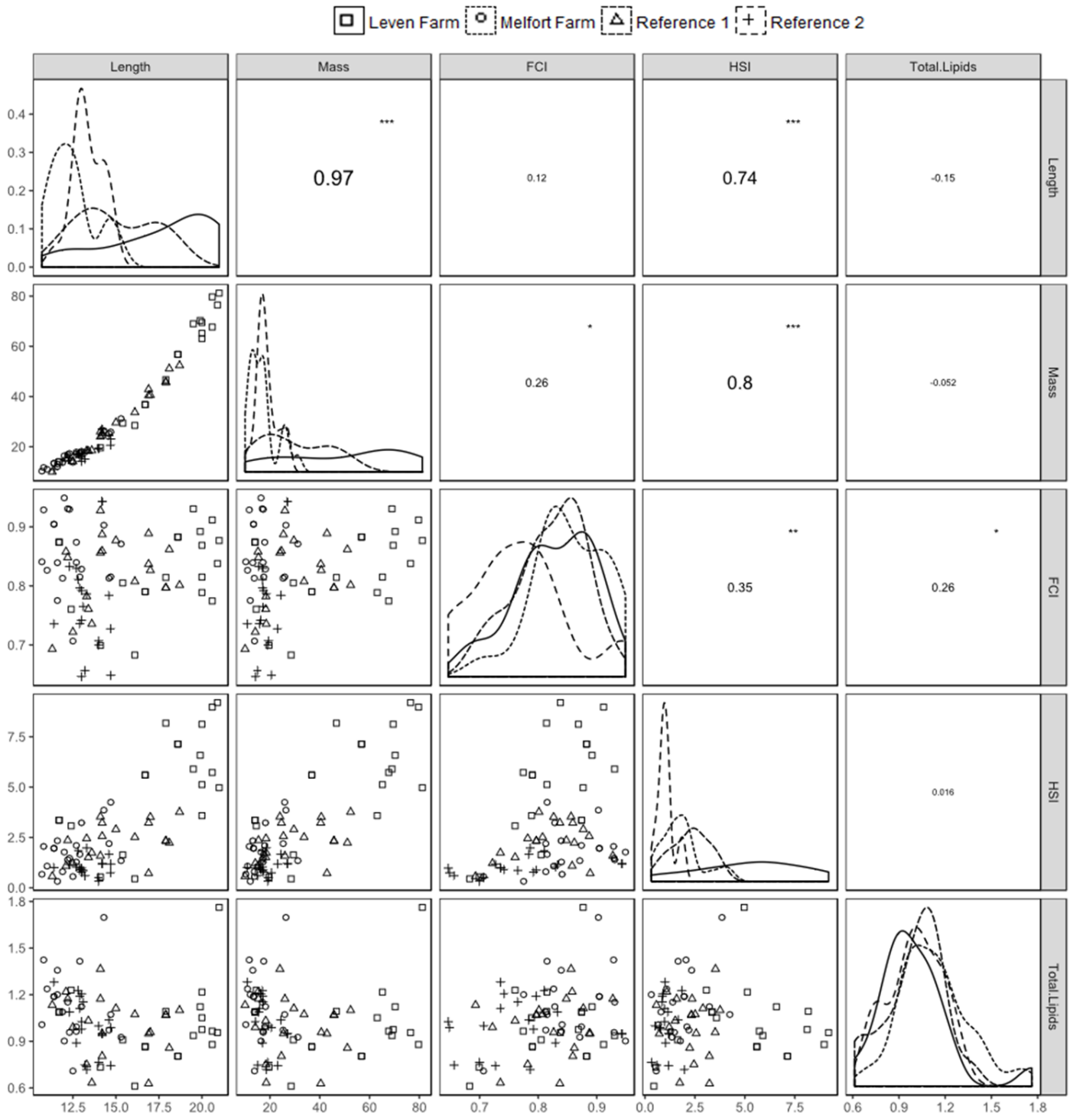

Figure 5.4 Scatter plots of length (cm), mass (g), FCI, HSI, and total lipid contents (%) in muscle tissues of whiting sampled near a salmon farm (Leven Farm), a halibut farm (Melfort Farm) and two reference sites (Reference 1 and 2). Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient is proportional to the significance level.

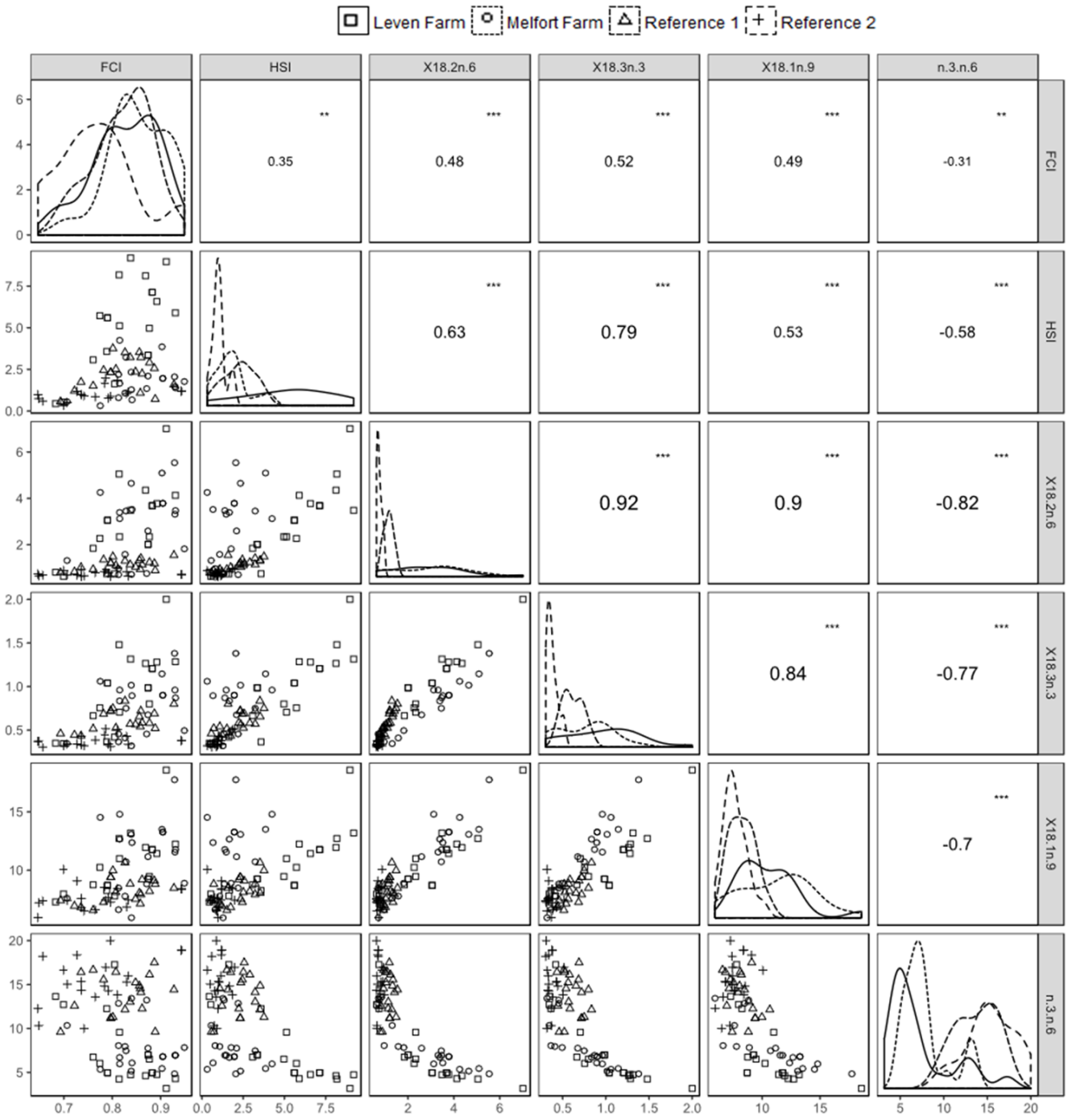

Figure 5.5 Scatter plots of FCI, HSI, and selected FAs (18:2n-6, 18:3n-3, 18:1n-9) and n-3/n-6 ratio in the muscle of whiting caught near a salmon farm (Leven Farm), a halibut farm (Melfort Farm) and two reference sites (Reference 1 and 2). Diagonal plots are density plots. Squares above the diagonal plots contain Pearson correlation coefficient (r) and significance level (0: ***, 0.001: **, 0.01: *). The font size of the correlation coefficient is proportional to the significance level.

5.3.3 Stomach contents

Stomach content analysis for both mackerel and whiting sampled near and away from the sea cages is presented in Figure 5.6A and B, respectively. Stomach content analysis was performed on all fish reported in Tables 5.1 and 5.2. Some pictures of waste pellets found in the stomachs of mackerel and whiting can be found in Appendix A.

5.3.3.1 Mackerel

Of the mackerel caught near both fish farms 7% had empty stomachs and of those caught away 16% had empty stomachs. Fish (clupeids) was the main item found in most of the stomachs of mackerel sampled near the two fish farms and reference sites (Figure 5.6A). About 10% of the mackerel sampled near the sea cages had consumed waste pellets and none were found in fish from reference sites (see Appendix A). Majority of the stomach contents from mackerel collected at Reference 3 was difficult to identify because digestion was at its final stages. The reason for this is that fish may have been stored on ice and/or limited amount of ice for longer period prior to collection.

5.3.3.2 Whiting

Of the whiting caught near both fish farms 17% had empty stomachs and of those caught away 40% had empty stomachs. Invertebrates were the main item found in most of the stomachs of whiting sampled near the sea cages and reference sites (Figure 5.6B). Of the whiting caught near the sea cages 31% had consumed waste pellets and none were found in whiting caught at reference sites (see also Appendix A). Information on diet of whiting from the third reference site can be found in Appendix C.

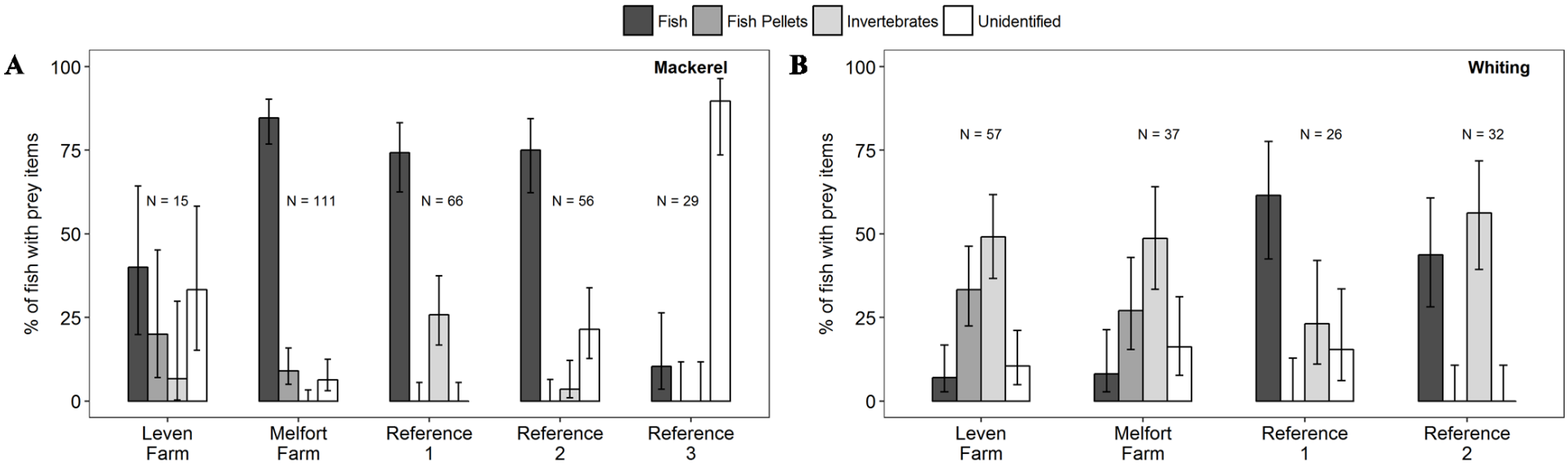

Figure 5.6 Stomach contents of mackerel (A) and whiting (B) sampled near a halibut, a salmon farm and at reference sites. Bars are drawn with 95% confidence intervals. N is the number of fish with non-empty stomachs.

5.3.4 Length, mass and condition

Descriptive statistics for length, mass and condition indices for both mackerel and whiting sampled near and away from sea cages are presented in Tables 5.1 and 5.2. Results for the linear mixed effect models are presented in Tables 5.3 and 5.4. Diagnostic plots for all models can be found in Appendix C.

5.3.4.1 Mackerel

Total length of mackerel near both fish farms was statistically different (Farm vs Reference difference: 4.8, 95% CI: [0.375, 9.23], t = 2.81, p = 0.04) than the length of mackerel sampled from reference sites (Table 5.3). The mass of mackerel near both fish farms was statistically different (Farm vs Reference difference: 133.8, 95% CI: [63.2, 204], t = 5.33, p = 0.006) than the mass of mackerel sampled at the three reference sites (Table 5.3). The effect of the farm on the length and mass of mackerel sampled near the sea cages appears to be stonger than the natural variability among sites (Table 5.3). The residual term had larger standard deviation than the standard deviation of the random effect which indicates some variability not explained by the model (Table 5.3). No significant differences in FCI (Farm vs Reference difference: 0.1, 95% CI: [-0.012, 0.14], t = 2.14, p = 0.08) and HSI (Farm vs Reference difference: -0.2, 95% CI [-1.69, 1.35], t = -0.29, p = 0.8) were found between mackerel sampled near and away from the sea cages (Table 5.3).

The diagnostic plots for the linear mixed effects models for the length, mass and FCI of mackerel indicated moderate levels of heterogeneity of variances (Figure D.1).

The diagnostic plots for linear mixed effect models for length, mass, FCI and HSI indicated some tailing (Figure C.1). Some of the assumptions (lack of homogeneity of variance) were moderately violated for the mass and FCI models of mackerel (Figure C.1).

5.3.4.2 Whiting

There were no statistical differences in length (Farm vs Reference difference: 1, 95% CI [-5.54, 7.48], t = 0.41, p = 0.7) of whiting sampled near and away from the two fish farms (Table 5.4). No statistical differences were detected in the mass (Farm vs Reference difference: 9.3, 95% CI [-32.9, 51.6], t = 0.61, p = 0.6) of whiting sampled

near and away from sea cages (Table 5.4). No significant differences in FCI (Farm vs Reference difference: 0, 95% CI: [-0.006, 0.0849], t = 0.51, p = 0.6) and HSI (Farm vs Reference difference: 1.5, 95% CI: [-2.36, 5.34], t = 1.07, p = 0.3) were found between whiting sampled near and away from the two fish farms (Table 5.4). There appears to be high variability in length, mass and HSI of whiting among the different sites (Table 5.4). The standard deviation of the random intercept was similar to that of the standard deviation of the residuals (Table 5.4).

The diagnostic plots of the linear mixed effect models for the length, mass and HSI of whiting sampled near and away from sea cages indicated some tailing and lack of homogeneity of variances (length, FCI and HSI) (Figure C.2). As for the mackerel data no outliers were removed from the data to minimise the bias about the ecological process taking place in the wild. Transformations of the response variables did not achieve the removal of the tailing in the models. The linear mixed effect models were kept as they provided sufficient information about the whiting sampled near the sea cages.

Table 5.1 Number of fish, length, mass, Fulton's condition index (FCI) and hepatosomatic index (HSI) for mackerel sampled near two fish farms and three reference sites. 95% confidence interval estimates of the sample means are presented.

	Melfort Farm	**Leven Farm**	**Reference Mackerel 1**	**Reference Mackerel 2**	**Reference Mackerel 3**
No. of fish	110	17	69	67	45
Length (cm)	29.9 [28.53, 31.23]	33.1 [30.70, 35.48]	24.0 [22.88, 25.17]	26.3 [25.61, 26.97]	29.0 [28.55, 29.45]
Mass (g)	261 [232.89, 290.05]	336 [253.36, 418.61]	124 [108.58, 140.25]	157 [145.04, 168.92]	181 [170.50, 192.19]
FCI	0.83 [0.82, 0.90]	0.86 [0.82, 0.87]	0.78 [0.77, 0.79]	0.83 [0.82, 0.85]	0.73 [0.72, 0.75]
HSI	2.35 [2.20, 2.49]	1.73 [1.53, 1.93]	2.15 [2.01, 2.89]	3.21 [3.05, 3.38]	1.27 [1.20, 1.34]

Table 5.2 Number of fish, length, mass, Fulton's condition index (FCI) and hepatosomatic index (HSI) for whiting sampled near two fish farms and two reference sites. 95% confidence interval estimates of the sample means are presented.

	Melfort Farm	**Leven Farm**	**Reference Whiting 1**	**Reference Whiting 2**
No. of fish	41	54	40	55
Length (cm)	12.2 [11.77, 12.55]	18.2 [17.37, 19.09]	15.6 [14.80, 16.35]	12.9 [12.62, 13.19]
Mass (g)	15 [13.81, 16.93]	55 [48.09, 61.28]	35 [29.63, 39.48]	17 [15.88, 18.14]
FCI	0.83 [0.81, 0.85]	0.83 [0.81, 0.84]	0.85 [0.83, 0.87]	0.78 [0.76, 0.80]
HSI	1.55 [1.22, 2.95]	5.19 [4.40, 5.98]	2.63 [2.30, 2.95]	1.17 [1.03, 1.31]

Table 5.3 Linear mixed effects models summary table for length (cm), mass (g), FCI and HSI of mackerel sampled near two fish farms and three reference sites. ***Note:*** SE: standard error, df: degrees of freedom, significant level: P < 0.05, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Length (cm)	Intercept	31.209	1.348	5.114	23.148	< 0.000	Intercept (Location)	2.866	1.693
	Treatment	-4.805	1.712	4.893	-2.807	0.039	Residual	25.948	5.094
Mass (g)	Intercept	286.919	19.965	4.014	14.371	<0.000	Intercept (Location)	502.6	22.42
	Treatment	-133.769	25.104	3.882	-5.329	<0.006	Residual	11295.3	106.28
FCI	Intercept	0.847	0.023	5.391	36.287	<0.0000	Intercept (Location)	0.001	0.031
	Treatment	-0.064	0.030	5.204	-2.144	0.083	Residual	0.004	0.066
HSI	Intercept	2.044	0.461	5.128	4.434	0.006	Intercept (Location)	0.411	0.641
	Treatment	0.170	0.593	5.066	0.286	0.786	Residual	0.414	0.643

Table 5.4 Linear mixed effects models summary table for length (cm), mass (g), FCI and HSI of whiting sampled near two fish farms and two reference sites. ***Note:*** SE: standard error, df: degrees of freedom, significant level: $P < 0.05$, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Length (cm)	Intercept	15.205	1.658	4.00	9.170	0.001	Intercept (Location)	5.398	2.323
	Treatment	-0.968	2.345	4.00	-0.413	0.701	Residual	4.684	2.164
Mass (g)	Intercept	35.086	10.763	4.000	3.260	0.031	Intercept (Location)	226.8	15.06
	Treatment	-9.332	15.221	4.000	-0.613	0.573	Residual	226.3	15.04
FCI	Intercept	0.829	0.018	3.983	45.418	0.000	Intercept (Location)	0.001	0.024
	Treatment	-0.013	0.026	3.986	-0.508	0.639	Residual	0.005	0.070
HSI	Intercept	3.381	0.980	4.001	3.450	0.026	Intercept (Location)	1.856	1.362
	Treatment	-1.488	1.385	3.996	-1.074	0.343	Residual	2.920	1.709

5.3.5 Total lipids and fatty acid composition

The lipid and FA analysis of the diets fed to farmed fish in both farms can be found in Table 5.5. Lipid content and levels of selected FAs for mackerel and whiting sampled near the two fish farms and at reference sites can be found in Tables 5.6 and 5.7, respectively. Full FA profiles for commercial diets, mackerel and whiting fillets can be found in Appendix C. Diagnostic plots for all linear mixed effect models applied to the lipid and FA data can be found in Appendix C.

5.3.5.1 Commercial diets

Information on the composition of the salmon diet used in 2014 was provided by staff members at the farm. The analytical constituents of the salmon diet were: oils and fats (23.9%), protein (42.4%), fibre (2.5%), ash (7.9%), phosphorous (1.4%), calcium (1.0%), and sodium (1.0%). The composition of the diet was: fish meal, horse beans, soya (bean) protein concentrate, fish oil, vegetable oil (rape), sunflower ext, maize gluten, distillers dark grains, mono-ammonium phosphate, and grain flour. I was unable to obtain information on the halibut diet for the year 2014.

The proportion of total lipid in commercial fish feeds used in the halibut and salmon farms in 2014 was 25.6% (Table 5.5). The diet at the salmon farm was rich in terrestrially based oils such as 18:2n-6, 18:3n-3 whereas the diet at the halibut was rich in marine oils such as 22:6n-3 (Table 5.5). The halibut diet used in 2014 was also rich in 20:1n-9 and 22:1n-11 which are indicators for copepods (Iverson 2009) (Table 5.5).

The diet used for the halibut farm in 2013 was very different than the one offered at the same farm in 2014 (Table 5.5). The diet at the halibut farm in 2013 was rich in SFAs and n-3 PUFAs when compared to the diet in 2014 (Table 5.5). Out of the three diets the halibut diet used in 2013 was the richest in fish oils whereas the diet at the salmon farm in 2014 was richest in vegetable oils (Table 5.5).

5.3.5.2 Mackerel

Total lipids of muscle tissues of mackerel sampled near sea cages did not statistically differ (Farm vs Reference difference: 0.0, 95% CI: [-0.035, 0.022], t = -0.64, p = 0.60) from the total lipids in mackerel sampled from reference sites (Table 5.8). Fatty acids that were found statistically different between the muscle tissues of mackerel

sampled near and away from sea cages were: 16:0 (Farm vs Reference difference: -1.1, 95% CI: [-1.88, -0.243], t = -2.58, p = 0.01), SFAs (Farm vs Reference difference: -1.7, 95% CI: [-2.48, -0.852], t =- 4.06, p = 0.000), 18:2n-6 (Farm vs Reference difference: 0.8, 95% CI: [0.21, 1.3], t = 2.75, p = 0.007), and n-6 PUFAs (Farm vs Reference difference: 0.6, 95%CI: [0.0379, 1.15], t = 2.12, p = 0.04) (Table 5.8). The differences in selected FAs in mackerel tissues near and away from sea cages appear to be related to the farm presence rather than the variability between sites (Table 5.8).

The diagnostic plots for the linear mixed effects models for the total lipids and most FAs indicated moderate violations of the assumptions including lack of homogeneity of variances and stong skewing in some of the Q-Q plots (see Figure C.3). Lack of homogeneity of variances and/or skewing in the distribution plots was noted for the total lipids, 14:0, 18:0, SFAs, 16:1n-7, 18:1n-7, 20:1n-9, 22:1n-11, MUFAs, 18:2n-6, 20:4n-6, total 6-PUFAs, 18:3n-3, 18:4n-3, 22:5n-3, 18:4n-3, total n-3 PUFAs, total PUFAs, n-3/n-6 (Figure C.3). Although, no observations were excluded as outliers. These observations indicated that the fish farms have a differential impact on the fish sampled near the sea cages. The linear mixed effects models were kept as the final choice because they gave enough information on the impact of the two fish farms on the fish sampled near its vicinity.

5.3.5.3 Whiting

The lipid content of muscle tissues of whiting sampled near the two fish farms did not statistically differ to the total lipid content in muscle tissues of whiting sampled at the reference sites (Farm vs Reference difference: 0.1, 95% CI: [-0.029, 0.170], t = 1.4, p = 0.2] (Table 5.9). Fatty acids that were found statistically different between the muscle tissues of mackerel sampled near and away from sea cages were: 18:1n-9 (Farm vs Reference difference: 3, 95% CI: [1.95, 3.98], t = 5.83, p = 0.000), MUFAs (Farm vs Reference difference: 4.8, 95% CI: [3.47, 6.11], t = 7.22, p = 0.000), 18:2n-6 (Farm vs Reference difference: 1.9, 95% CI: [1.35, 2.41], t = 7.1, p = 0.000), 20:4n-6 (Farm vs Reference difference: 0.6, 95% CI: [0.427, 0.869], t = 5.84, p = 0.000), 18:3n-3 (Farm vs Reference difference: 0.4, 95% CI: [0.053, 0.663], t = 3.26, p = 0.03), Total n-6 PUFAs (Farm vs Reference difference: 3.2, 95% CI: [2.45, 3.87], t = 8.86, p = 0.000), 22:5n-3 (Farm vs Reference difference: 1.3, 95% CI: [0.865, 1.790], t = 8.02, p = 0.001), 22:6n-3 (Farm vs Reference difference: -9.8, 95% CI: [-18, -1.68], t = -3.35, p = 0.03), n-3

PUFAs (Farm vs Reference difference: -6.8, 95% CI [-9.68, -3.99], t = -6.65, p = 0.003), Total PUFAs (Farm vs Reference difference: -3.7, 95% CI: [-6.21, -1.16], t = -4.04, p = 0.02) n-3/n-6 (Farm vs Reference difference: -6.8, 95% CI: [-8.19, -5.43], t = -9.82, p = 0.000) (Table 5.9). The differences in selected FAs in whiting tissues sampled near and far from sea cages appear to be related to the farm presence rather than the variability between sites (Table 5.9).

The diagnostic plots for the linear mixed effects models for the total lipids and FAs for whiting indicated moderate level of heterogeneity of variances and some skeweness in the distributions (Figure C.4). Heterogeneity of variances and/or tailing in the Q-Q plots could be noted in the FAs 18:4n-3, total n-3 PUFAs, total PUFAs, n-3/n-6, SFAs, 22:1n-11, 18:2n-6, 20:4n-6, total n-6 PUFAs, 18:3n-3, 20:5n-3, 22:5n-3, and n-3/n-6 (Figure C.4). As for the mackerel data no outliers were removed and no other models were used. The linear mixed effect models were sufficient to describe the impact of the two fish farms on the whiting sampled near the sea cages.

Table 5.5 Total lipid content (%) and fatty acid composition (%) of commercial diets used at Melfort and Leven farms. The commercial diet used at the halibut farm in 2013 is also presented in the table. Data are presented as means and 95% confidence intervals.

Location	Melfort halibut diet 2013	Melfort halibut diet 2014	Leven salmon diet 2014
Total Lipid	21.19 [21.16, 21.21]	25.58 [25.28, 25.88]	25.63 [23.67, 27.58]
Fatty Acids			
14:0	7.09 [6.77, 7.40]	4.95 [0.38, 9.52]	3.27 [2.89, 3.65]
16:0	18.35 [16.83, 19.87]	13.84 [7.49, 20.19]	11.92 [10.78, 13.06]
18:0	3.66 [3.28, 4.04]	2.43 [2.23, 2.62]	3.33 [3.33, 3.33]
Total SFAs	30.02 [28.06, 31.99]	21.99 [10.74, 33.23]	19.44 [17.92, 20.96]
16:1n-7	7.64 [6.30, 8.97]	4.56 [2.27, 6.85]	3.33 [2.95, 3.71]
18:1n-9	12.94 [12.11, 13.76]	19.33 [14.63, 24.03]	36.63 [34.34, 38.92]
18:1n-7	2.77 [2.45, 3.08]	2.91 [1.57, 4.24]	2.97 [2.84, 3.10]
20:1n-9	1.75 [1.68, 1.81]	7.35 [6.52, 8.17]	1.72 [0.96, 2.48]
22:1n-11	2.10 [1.72, 2.48]	11.01 [9.54, 12.47]	0.93 [0.73, 1.12]
Total MUFAs	28.32 [25.78, 30.86]	48.51 [45.33, 51.69]	46.84 [44.30, 49.38]
18:2n-6	7.22 [7.03, 7.42]	7.38 [6.11, 8.65]	13.22 [11.88, 14.55]
20:4n-6	0.97 [0.90, 1.03]	0.45 [0.32, 0.58]	0.35 [0.28, 0.41]
Total n-6 PUFAs	8.95 [8.37, 9.52]	8.50 [6.72, 10.28]	13.99 [12.85, 15.13]
18:3n-3	1.09 [0.89, 1.28]	1.92 [0.96, 2.87]	5.14 [4.82, 5.45]
18:4n-3	2.11 [1.86, 2.36]	2.05 [1.47, 2.62]	1.14 [1.01, 1.27]
20:5n-3	13.56 [12.29, 14.83]	5.89 [4.42, 7.35]	5.93 [4.72, 7.13]
22:5n-3	1.70 [1.38, 2.01]	0.99 [0.61, 1.37]	0.72 [0.52, 0.91]
22:6n-3	9.58 [7.67, 11.49]	8.53 [5.99, 11.07]	4.79 [3.77, 5.81]
Total n-3 PUFAs	28.66 [24.58, 32.72]	20.16 [13.87, 26.44]	18.04 [15.18, 20.89]
Total PUFAs	41.66 [37.14, 46.17]	29.50 [21.50, 37.50]	33.72 [29.65, 37.79]
n-3/n-6	3.20 [2.95, 3.45]	2.37 [2.14, 2.60]	1.29 [1.19, 1.39]

Table 5.6 Total lipid content (%) and relative fatty acid concentration (%) in muscle of mackerel sampled near two fish farms and three reference sites. Data are expressed as means and 95% confidence intervals.

Location	Melfort Farm	Leven Farm	Reference Mackerel 1	Reference Mackerel 2	Reference Mackerel 3
No. of fish	22	17	17	17	17
Total Lipid	6.67 [4.23, 9.10]	7.17 [5.10, 9.24]	6.06 [4.27, 7.85]	6.93 [5.41, 8.46]	9.71 [7.15, 12.28]
Fatty Acids					
14:0	3.94 [3.55, 4.32]	3.57 [3.14, 4.00]	4.51 [4.24, 4.79]	3.58 [3.29, 3.88]	4.74 [4.38, 5.10]
16:0	17.77 [17.13, 18.41]	17.81 [16.99, 18.63]	18.47 [17.13, 19.01]	19.66 [19.13, 20.18]	18.41 [17.73, 19.09]
18:0	4.44 [4.17, 4.71]	4.61 [4.25, 4.98]	4.43 [4.19, 4.66]	4.65 [4.48, 4.81]	4.14 [3.86, 4.41]
Total SFA	26.92 [26.27, 27.57]	26.60 [25.65, 27.55]	28.38 [27.86, 28.91]	28.71[28.26, 29.16]	28.25 [27.61, 28.90]
16:1n-7	3.82 [3.59, 4.06]	4.04 [3.76, 4.32]	3.87 [3.65, 4.08]	3.99 [3.87, 4.11]	3.91 [3.75, 4.06]
18:1n-9	16.37 [14.48, 18.27]	18.61 [16.30, 20.92]	14.97 [13.80, 16.14]	19.34 [18.16, 20.51]	17.19 [15.61, 18.77]
18:1n-7	3.51 [3.20, 3.81]	3.75 [3.47, 4.02]	3.74 [3.51, 3.96]	4.54 [4.39, 4.97]	3.96 [3.67, 4.26]
20:1n-9	5.28 [4.62, 5.94]	4.50 [3.85, 5.16]	4.74 [4.18, 5.30]	3.86 [3.52, 4.21]	5.37 [4.71, 6.03]
22:1n-11	8.16 [6.62, 9.68]	6.00 [4.72, 7.28]	6.83 [5.56, 8.10]	4.94 [4.12, 5.76]	7.77 [6.45, 9.08]
Total MUFAs	40.23 [38.24, 42.22]	39.67 [37.59, 41.75]	37.11 [35.76, 38.45]	39.09 [38.35, 39.82]	40.94 [39.75, 42.14]
18:2n-6	1.90 [1.46, 2.33]	2.32 [1.46, 3.17]	1.34 [1.22, 1.46]	1.17 [1.08, 1.29]	1.45 [1.36, 1.52]
20:4n-6	1.15 [1.03, 1.28]	0.81 [0.70, 0.93]	1.16 [1.03, 1.29]	0.96 [0.88, 1.03]	1.10 [0.97, 1.23]
Total n-6 PUFA	4.20 [3.76, 4.65]	3.96 [3.16, 4.75]	3.71 [3.44, 3.97]	3.14 [2.97, 3.31]	3.66 [3.46, 3.87]
18:3n-3	1.00 [0.88, 1.11]	1.17 [0.91, 1.42]	0.97 [0.90, 1.04]	0.85 [0.76, 0.93]	1.05 [0.95, 1.14]
18:4n-3	1.69 [1.49, 1.89]	1.85 [1.54, 2.16]	2.02 [1.85, 2.18]	1.74 [1.58, 1.90]	2.19 [1.91, 2.47]
20:5n-3	6.54 [6.13, 6.95]	8.09 [7.61, 8.58]	7.06 [6.66, 7.46]	7.20 [6.86, 7.54]	6.03 [5.71, 6.34]
22:5n-3	1.57 [1.42, 1.72]	1.63 [1.55, 1.71]	1.52 [1.44, 1.59]	1.53 [1.47, 1.58]	1.32 [1.26, 1.39]
22:6n-3	15.80 [14.30, 17.31]	15.09 [13.21, 16.98]	16.98 [16.06, 17.90]	15.73 [15.18, 16.27]	14.35 [13.72, 14.99]
Total n-3 PUFA	27.52 [25.89, 29.15]	28.69 [26.90, 30.48]	29.60 [28.52, 30.67]	27.99 [27.24, 28.74]	26.00 [25.08, 26.92]
Total PUFA	32.85 [31.19, 34.51]	33.73 [32.24, 35.21]	34.51 [33.43, 35.59]	32.20 [31.41, 33.00]	30.81 [29.82, 31.79]
n-3/n-6	7.36 [6.53, 8.20]	9.23 [7.92, 10.54]	8.37 [7.63, 9.11]	9.13 [8.58, 9.69]	7.23 [6.83, 7.64]

Table 5.7 Total lipid content (%) and relative fatty acid concentration (%) in muscle of whiting sampled near two fish farms and two reference sites. Data are expressed as means and 95% confidence intervals.

	Melfort Farm	**Leven Farm**	**Reference Whiting 1**	**Reference Whiting 2**
No. of fish	19	17	19	17
Total Lipid	1.13 [1.01, 1.24]	1.01 [0.88, 1.14]	1.00 [0.90, 1.09]	1.01 [0.92, 1.09]
Fatty Acids				
14:0	1.05 [0.98, 1.13]	1.32 [1.20, 1.43]	1.00 [0.93, 1.06]	0.95 [0.89, 1.01]
16:0	15.84 [15.42, 16.39]	16.93 [16.38, 17.48]	18.38 [18.08, 18.85]	17.01 [16.59, 17.57]
18:0	5.75 [5.49, 5.98]	5.98 [5.74, 6.22]	5.89 [5.77, 5.99]	5.82 [5.67, 5.98]
Total SFA	23.12 [22.53, 23.83]	24.71 [24.07, 25.35]	25.74 [25.39, 26.23]	24.20 [23.68, 24.87]
16:1n-7	2.08 [1.98, 2.19]	1.91 [1.79, 2.03]	1.83 [1.70, 1.96]	1.34 [1.28, 1.39]
18:1n-9	11.12 [9.99, 12.06]	10.62 [9.66, 11.58]	8.23 [7.88, 8.63]	7.59 [7.21, 7.99]
18:1n-7	3.25 [3.15, 3.36]	3.41 [3.31, 3.51]	3.18 [3.04, 3.35]	2.57 [2.38, 2.81]
20:1n-9	1.63 [1.42, 1.83]	1.29 [1.14, 1.44]	0.85 [0.76, 0.90]	1.35 [1.22, 1.44]
22:1n-11	0.91 [0.73, 1.10]	0.52 [0.43, 0.61]	0.44 [0.36, 0.49]	0.57 [0.43, 0.68]
Total MUFAs	20.84 [19.50, 22.02]	19.82 [18.57, 21.07]	16.04 [15.44, 16.64]	15.08 [14.43, 15.73]
18:2n-6	2.84 [2.30, 3.31]	2.84 [2.24, 3.44]	1.15 [1.09, 1.24]	0.76 [0.72, 0.81]
20:4n-6	2.50 [2.33, 2.65]	2.41 [2.26, 2.55]	1.78 [1.56, 1.92]	1.84 [1.65, 1.96]
Total n-6	6.62 [6.06, 7.07]	7.50 [6.63, 8.37]	3.95 [3.70, 4.11]	3.80 [3.47, 4.02]
PUFAs				
18:3n-3	0.75 [0.64, 0.85]	0.96 [0.80, 1.12]	0.61 [0.58, 0.65]	0.38 [0.35, 0.40]
18:4n-3	0.51 [0.47, 0.58]	0.48 [0.43, 0.53]	0.99 [0.92, 1.09]	0.45 [0.43, 0.48]
20:5n-3	12.85 [12.30, 13.45]	15.19 [14.58, 15.80]	14.25 [13.44, 15.17]	10.83 [10.50, 11.25]
22:5n-3	2.32 [2.16, 2.44]	2.79 [2.54, 3.04]	1.22 [1.15, 1.26]	1.23 [1.14, 1.31]
22:6n-3	31.24 [30.21, 32.41]	26.64 [24.28, 29.00]	35.35 [34.06, 36.47]	42.27 [41.35, 43.07]
Total n-3	48.24 [47.03, 49.59]	46.99 [45.10, 48.88]	53.19 [52.20, 54.14]	55.80 [54.75, 56.81]
PUFAs				
Total PUFAs	56.04 [55.02, 57.10]	55.47 [54.16, 56.77]	58.22 [57.24, 59.07]	60.72 [59.53, 61.76]
n-3/n-6	7.85 [7.02, 8.84]	7.50 [6.10, 8.91]	13.87 [13.14, 14.84]	15.23 [14.46, 16.34]

Table 5.8 Linear mixed effects models summary table for total lipid and fatty acids of muscle tissues in mackerel sampled near two fish farms and three reference sites. ***Note:*** SE: standard error, df: degrees of freedom, significant level: P < 0.05, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Total Lipids	Intercept	0.007	0.008	3.952	8.615	0.000	Intercept (Location)	0.000	0.005
	Treatment	0.007	0.011	4.344	0.640	0.555	Residual	0.002	0.044
14:0	Intercept	3.763	0.277	4.518	13.578	0.000	Intercept (Location)	0.094	0.306
	Treatment	0.516	0.362	4.721	1.426	0.216	Residual	1.155	1.075
16:0	Intercept	17.786	0.310	90.000	57.417	0.000	Intercept (Location)	0.000	0.000
	Treatment	1.060	0.412	90.000	2.577	0.012	Residual	3.742	1.934
18:0	Intercept	4.516	0.129	90.00	34.988	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.113	0.172	90.00	-0.662	0.510	Residual	0.650	0.806
SFAs	Intercept	26.781	0.309	90.00	86.665	0.000	Intercept (Location)	0.000	0.000
	Treatment	1.668	0.411	90.00	4.063	0.000	Residual	0.000	0.000
16:1n-7	Intercept	3.915	0.100	90.00	39.124	0.000	Intercept (Location)	0.000	0.000
	Treatment	0.004	0.133	90.00	0.032	0.975	Residual	0.391	0.625
18:1n-9	Intercept	17.404	1.052	4.314	16.541	0.000	Intercept (Location)	0.839	0.916
	Treatment	-0.239	1.382	4.6440	-0.173	0.870	Residual	26.640	5.161
18:1n-7	Intercept	3.619	0.187	4.416	19.370	0.000	Intercept (Location)	0.037	0.193
	Treatment	0.462	0.244	4.659	1.890	0.122	Residual	0.630	0.794
20:1n-9	Intercept	4.922	0.364	4.304	13.508	0.000	Intercept (Location)	0.098	0.313
	Treatment	-0.264	0.479	4.639	-0.552	0.607	Residual	3.244	1.801
22:1n-11	Intercept	7.165	0.779	4.391	9.195	0.000	Intercept (Location)	0.420	0.648
	Treatment	-0.653	1.024	4.748	-0.638	0.553	Residual	15.416	3.926
MUFAs	Intercept	39.986	0.791	3.673	50.559	0.000	Intercept (Location)	0.036	0.190
	Treatment	-0.941	1.050	4.158	-0.896	0.419	Residual	23.678	4.886
18:2n-6	Intercept	2.079	0.207	90.00	10.028	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.758	0.275	90.00	-2.751	0.007	Residual	1.676	1.295
20:4n-6	Intercept	0.992	0.089	4.817	11.098	0.000	Intercept (Location)	0.010	0.100

	Treatment	0.081	0.117	5.038	0.692	0.520	Residual	0.123	0.350
Total n-6 PUFAs	Intercept	4.095	0.210	90.00	19.483	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.593	0.279	90.00	-2.122	0.037	Residual	1.723	1.313
18:3n-3	Intercept	1.072	0.065	90.00	16.386	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.114	0.087	90.00	-1.315	0.192	Residual	0.000	0.000
18:4n-3	Intercept	1.759	0.109	90.00	16.098	0.000	Intercept (Location)	0.000	0.000
	Treatment	0.228	0.145	90.00	1.535	0.128	Residual	0.466	0.682
20:5n-3	Intercept	7.300	0.450	4.898	16.240	0.000	Intercept (Location)	0.331	0.575
	Treatment	-0.536	0.583	4.994	-0.919	0.400	Residual	1.412	1.884
22:5n-3	Intercept	1.597	0.0478	3.69	33.381	0.000	Intercept (Location)	0.000	0.01
	Treatment	-0.142	0.064	4.191	-2.228	0.087	Residual	0.000	0.298
22:6n-3	Intercept	15.494	0.600	90.00	25.842	0.000	Intercept (Location)	0.000	0.000
	Treatment	0.192	0.192	90.00	0.241	0.810	Residual	14.02	3.744
Total n-3 PUFAs	Intercept	28.056	0.803	4.197	34.941	0.000	Intercept (Location)	0.460	0.678
	Treatment	-0.195	1.055	4.532	-0.185	0.861	Residual	16.085	4.011
Total PUFAs	Intercept	33.256	0.817	4.244	40.7	0.000	Intercept (Location)	0.557	0.747
	Treatment	-0.750	1.072	4.546	-0.700	0.518	Residual	15.074	3.883
n-3/n-6	Intercept	30.312	2.522	4.569	12.020	0.000	Intercept (Location)	5.760	2.400
	Treatment	2.332	3.304	4.870	0.706	0.513	Residual	134.660	11.600

Table 5.9 Linear mixed effect models summary table for total lipid and fatty acids of muscle tissues in whiting sampled near two fish farms and three reference sites. ***Note:*** SE: standard error, df: degrees of freedom, significant level: $P < 0.05$, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Total Lipids	Intercept	1.072	0.036	73.00	30.164	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.070	0.050	73.0	-1.404	0.164	Residual	0.000	0.000
14:0	Intercept	1.178	0.068	3.997	17.249	0.000	Intercept (Location)	0.007	0.081
	Treatment	-0.204	0.096	3.964	-2.117	0.102	Residual	0.050	0.223
16:0	Intercept	16.380	0.439	4.034	37.327	0.000	Intercept (Location)	0.286	0.535
	Treatment	1.321	0.619	4.034	2.132	0.099	Residual	1.776	1.333
18:0	Intercept	5.858	0.092	73.0	63.36	0.000	Intercept (Location)	0.000	0.000
	Treatment	-0.004	0.130	73.0	-0.030	0.976	Residual	0.308	0.555
SFAs	Intercept	23.906	0.554	4.023	43.163	0.000	Intercept (Location)	0.475	0.689
	Treatment	1.067	0.782	3.997	1.365	0.244	Residual	2.492	1.579
16:1n-7	Intercept	1.997	0.130	4.032	15.339	0.000	Intercept (Location)	0.029	0.169
	Treatment	-0.414	0.184	4.014	-2.249	0.088	Residual	0.096	0.309
18:1n-9	Intercept	10.884	0.362	73.0	30.05	0.000	Intercept (Location)	0.000	0.000
	Treatment	-2.965	0.509	73.0	-5.83	0.000	Residual	4.721	2.173
18:1n-7	Intercept	3.326	0.159	4.045	20.981	0.000	Intercept (Location)	0.039	0.199
	Treatment	-0.453	0.224	4.020	-2.026	0.112	Residual	0.193	0.440
20:1n-9	Intercept	1.461	0.151	4.03	9.685	0.001	Intercept (Location)	0.036	0.189
	Treatment	-0.364	0.213	4.01	-1.708	0.163	Residual	0.177	0.421
22:1n-11	Intercept	0.717	0.102	4.00	7.053	0.002	Intercept (Location)	0.014	0.117
	Treatment	-0.212	0.143	3.963	-1.479	0.214	Residual	0.127	0.356
MUFAs	Intercept	20.358	0.472	73.0	73.147	0.000	Intercept (Location)	0.000	0.000
	Treatment	-4.786	0.663	73.0	-7.221	0.000	Residual	8.015	2.831
18:2n-6	Intercept	2.842	0.189	73.0	15.076	0.000	Intercept (Location)	0.000	0.000
	Treatment	-1.882	0.265	73.0	-7.105	0.000	Residual	1.28	1.131
20:4n-6	Intercept	2.455	0.079	73.0	31.115	0.000	Intercept (Location)	0.000	0.000

	Treatment	-0.648	0.111	73.0	-5.843	0.000	Residual	0.224	0.474
Total n-6 PUFAs	Intercept	7.034	0.254	73.0	27.710	0.000	Intercept (Location)	0.000	0.000
	Treatment	-3.159	0.357	73.0	-8.859	0.000	Residual	2.320	1.523
18:3n-3	Intercept	0.852	0.078	4.04	10.934	0.000	Intercept (Location)	0.008	0.088
	Treatment	-0.358	0.110	4.00	-3.255	0.031	Residual	0.080	0.283
18:4n-3	Intercept	0.498	0.135	4.01	3.681	0.021	Intercept (Location)	0.035	0.187
	Treatment	0.227	0.191	4.01	1.187	0.301	Residual	0.030	0.174
20:5n-3	Intercept	14.014	1.038	4.014	13.501	0.000	Intercept (Location)	1.957	1.399
	Treatment	-1.470	1.467	4.004	-1.002	0.373	Residual	3.552	1.885
22:5n-3	Intercept	2.552	0.117	3.991	21.766	0.000	Intercept (Location)	0.017	0.130
	Treatment	-1.327	0.165	3.949	-8.021	0.001	Residual	0.192	0.438
22:6n-3	Intercept	28.956	2.082	4.018	13.911	0.000	Intercept (Location)	7.680	2.771
	Treatment	9.843	2.941	4.005	3.346	0.029	Residual	17.70	4.207
Total n-3 PUFAs	Intercept	47.640	0.730	4.111	65.222	0.000	Intercept (Location)	0.305	0.552
	Treatment	6.832	1.028	4.035	6.647	0.003	Residual	13.708	3.702
Total PUFAs	Intercept	55.764	0.649	4.11	85.938	0.000	Intercept (Location)	0.347	0.589
	Treatment	3.689	0.914	4.05	4.036	0.015	Residual	8.910	2.985
n-3/n-6	Intercept	7.684	0.494	73.0	15.562	0.000	Intercept (Location)	0.000	0.000
	Treatment	6.810	0.694	73.0	9.818	0.000	Residual	8.777	2.963

5.3.6 Linear discriminant analysis

Results of LDA for mackerel and whiting sampled near and away from sea cages can be found in Figures 5.7 and 5.8. Coefficients for the linear discriminant functions for the FA data for both mackerel and whiting can be found in Tables 5.10 and 5.11, respectively.

5.3.6.1 Mackerel

A linear discriminant function plot showed separation between mackerel sampled near and away from sea cages (Figure 5.7). The output of the linear discriminant analysis provided the coefficient for the linear discriminant functions. For example, the linear discriminant function 1 is: z = 2.63 × 14:0 + 0.62 × 16:0 + 1.25 × 18:0…. (Table 5.10). Taking the absolute value of the Coefficients and ranking them showed that the FAs that contributed to the most separation between mackerel sampled near and away from sea cages were: 18:3n-3, 18:1n-7, 14:0, and 18:0. The FAs 18:3n-3, 18:0, 14:0, 18:1n-7, and 20:5n-3 contributed to the separation between mackerel sampled near sea cages of the salmon and halibut farms (Table 5.10). Linear discriminant function correctly assigned 52.2% of all samples to their origin (Melfort Farm (50%), Leven Farm (77%), Reference Mackerel 1 (24%), Reference Mackerel 2 (65%) and Reference Mackerel 3 (47%)).

5.3.6.2 Whiting

A plot of all selected FAs split up into two axes showed that FA profiles of whiting sampled near sea cages are distinct from the FA profiles of fish caught away from cages (Figure 5.8A and B). Based on the coefficients for the linear discriminant functions the FAs that contributed most to the discrimination between whiting sampled near and away from sea cages were: 22:5n-3, 16:1n-7, 22:1n-11 and 18:2n-6 (Table 5.11). The FAs 18:4n-3, 20:1n-9, 14:0 and 18:3n-3 contribute to the discrimination between the two reference sites of whiting (Table 5.11). It is also worth noting that within the whiting sampled at Reference 1 site there appears to be two distinct groups (Figure 5.8A). The FAs 14:0, 18:3n-3, and 16:1n-7 contributed to the separation between whiting sampled near the halibut and salmon farm (Table 5.11). Linear discriminant analysis correctly assigned overall 90.4% of all samples (Melfort Farm (89.5%), Leven Farm (76.5%), Reference Whiting 1 (95%) and Reference Whiting 2 (100%)).

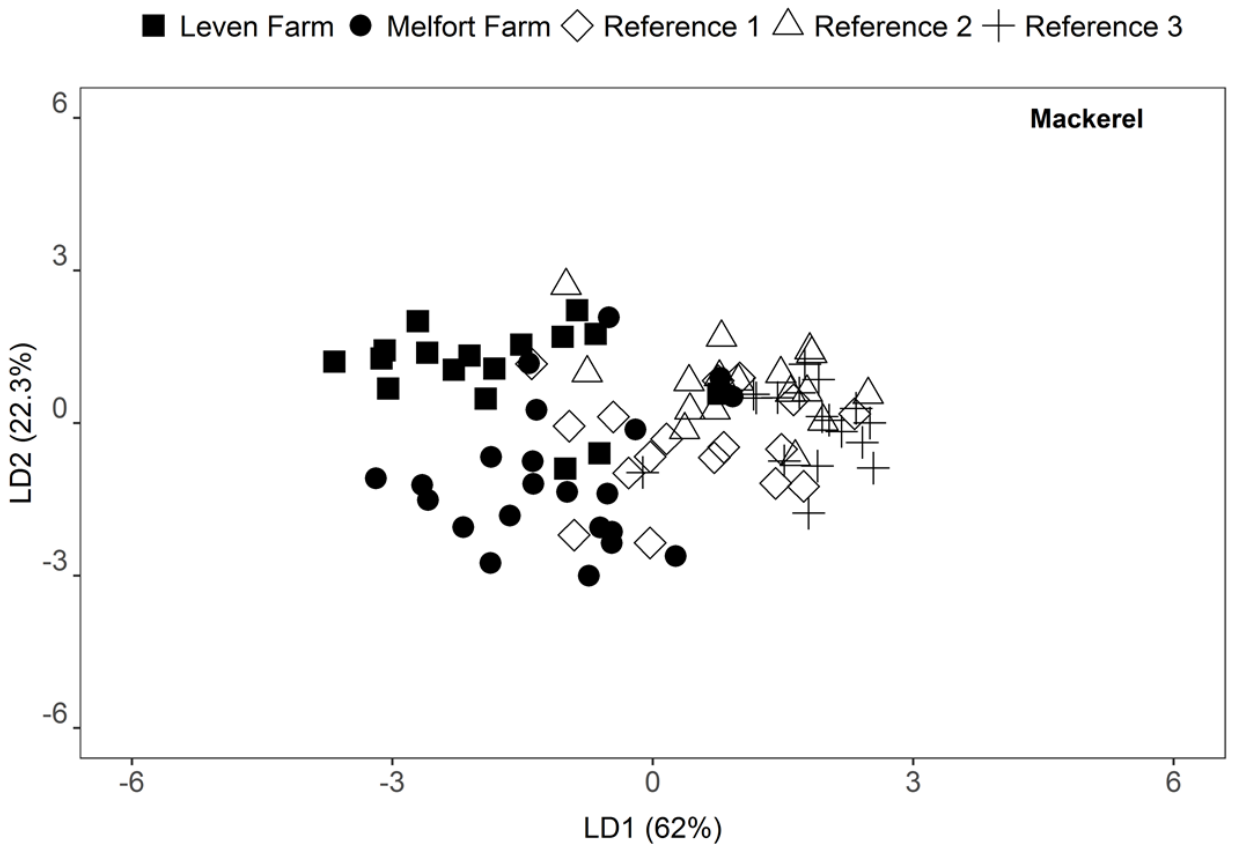

Figure 5.7 Linear discriminant analysis of fatty acid profile of mackerel sampled near two fish farms and three reference sites.

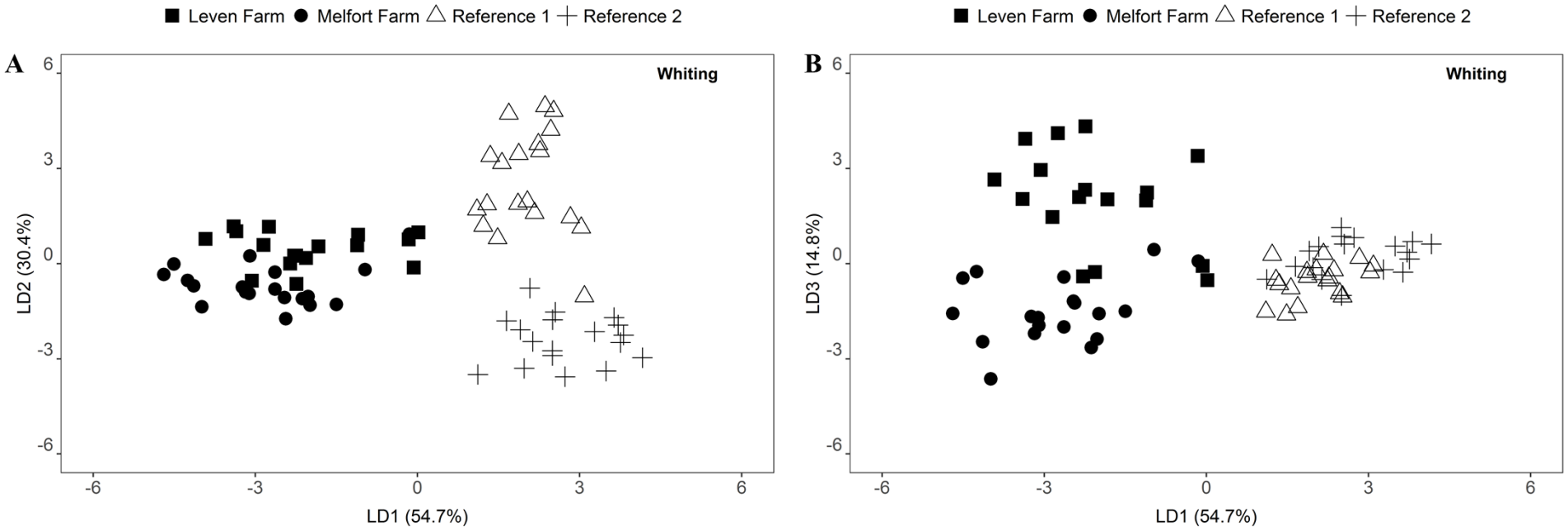

Figure 5.8 Linear discriminant analysis (A) LD1 vs LD2 and (B) LD1 vs LD3 of fatty acid profile for whiting sampled near two fish farms and two reference sites.

Table 5.10 Coefficient of the linear discriminant functions for the fatty acid data for mackerel.

Fatty Acids	Linear Discriminant Function 1	Linear Discriminant Function 2
14:0	2.63	1.61
16:0	0.62	1.02
18:0	1.25	2.12
16:1n-7	0.52	1.21
18:1n-9	0.61	0.89
18:1n-7	3.53	1.58
20:1n-9	0.23	1.04
22:1n-11	1.01	0.92
18:2n-6	-0.67	0.67
20:4n-6	0.92	0.31
18:3n-3	-5.18	3.01
18:4n-3	0.65	0.94
20:5n-3	0.26	1.46
22:5n-3	-0.26	-0.44
22:6n-3	0.79	0.86

Table 5.11 Coefficient of the linear discriminant functions for the fatty acid data for whiting.

Fatty Acids	Linear Discriminant Function 1	Linear Discriminant Function 2	Linear Discriminant Function 3
14:0	-0.47	2.52	6.24
16:0	1.09	0.33	0.51
18:0	0.83	0.15	0.53
16:1n-7	-2.83	-1.03	-1.82
18:1n-9	-1.11	-0.17	0.34
18:1n-7	-0.73	-0.92	-0.73
20:1n-9	0.04	-3.35	-0.93
22:1n-11	-2.12	0.96	-0.85
18:2n-6	-1.72	-0.64	0.30
20:4n-6	-1.24	0.69	-0.05
18:3n-3	0.69	2.25	1.69
18:4n-3	0.66	4.89	-1.47
20:5n-3	-1.01	-0.38	0.51
22:5n-3	-3.71	-0.88	1.43
22:6n-3	-0.96	-0.51	0.38

5.4 Discussion

Results of the present chapter build on the results of Chapter 4. Both mackerel and whiting sampled near the sea cages of the halibut and salmon farms consumed some of the waste feed which was detected by both stomach content and fatty acid analysis. The LDA was able to distinguish between mackerel and whiting sampled near the halibut and salmon farms and also the reference sites. The overall impacts of both the halibut farm and the salmon farm appear to be more evident in whiting than in mackerel.

5.4.1 Impacts of two fish farms (halibut and salmon) on mackerel

The commercial fish food that enters the sea cages is partitioned into farmed fish biomass and the release of dissolved organic and inorganic nutrients, particulate organic nutrients and the direct loss of feed (Olsen et al. 2008). Assuming a maximum of 5% waste feed (Gillibrand et al. 2002), during the sampling period of 2014, the amount of lost feed from the halibut and salmon farms was 336.4 kg and 10097.1 kg, respectively (Table 3.1; Chapter 3). As evident from the stomach content analysis of this study and the previous (Chapter 4) mackerel sampled near both fish farms consumed some of this waste feed.

Fish (mainly clupeids) were the main item consumed by mackerel sampled near the the sea cages. It is worth noting that schools of clupeids were not noticed during the sampling events around the salmon sea cages in Loch Leven. The mackerel sampled near the salmon cages had mainly fish (unidentifiable) in their stomachs and two of the fish appeared to have consumed some gadoid species based on the otoliths that were found in the stomachs. These gadoid species (e.g. saithe (Chapter 4), whiting (next section)) in turn consumed waste feed and/or other particulate organic matter and/or other marine organisms that may have consumed waste nutrients from the fish farms.

The majority of the fish found consumed by mackerel were clupeids. The clupeids possibly consumed zooplankton and/or particulate organic matter near the sea cages (see Appendix A). The release of dissolved nutrients may promote phytoplankton growth that may attract the zooplankton; however as discussed in Chapter 4 it is less likely to take place within Loch Melfort because of the hydrodynamics in the loch. In Loch Leven, the flushing time is three days which is less than the time for phytoplankton growth and biomass accumulation (Gowen and Ezzi 1992 cited in Mente et al. 2008). Therefore, there

is faster dilution of nutrient discharges from the salmon farm and less time for phytoplankton to grow in Loch Leven than in Loch Melfort.

It is worth noting that much lower numbers of mackerel were sampled in Loch Leven than in Loch Melfort during the fieldwork visits in 2014. This difference is not likely to be temperature related because the average temperature between the two lochs were similar (see Appendix A). The difference may be related to salinity differences between the two lochs: lower salinity in Loch Leven than in Loch Melfort (see Appendix A). However, this is only speculative.

Mackerel sampled near the sea cages of both farms were longer and heavier than those sampled from reference sites. Results were similar to those reported in Chapter 4. As discussed in Chapter 4, this may be related to the abundant food resources around the cages. Differences in length and mass of mackerel sampled near and away from sea cages may be age-related. Based on the length at age key (see Appendix A) the mackerel sampled near both fish farms ranged from 0-11 years whereas those sampled at the reference sites ranged from 0-5 years.

As in Chapter 4, no differences in FCI, HSI and total lipids were detected for mackerel sampled near both fish farms and at reference sites. The consumption of high energy food (e.g. fish) by mackerel sampled near and away from both fish farms may explain the lack of difference in FCI, HSI and total lipids (see also discussion in Chapter 4). During sampling in 2013 and 2014, some of the mackerel sampled near the sea cages appeared to be longer, heavier, and had higher total lipid levels in muscle tissues. Based on the length at age key (Appendix A) these fish might be between 4 and 11 years old. Mackerel grows rapidly in length until they reach sexual maturity, at an average age of 3 years, and then this increase in length decreases annually (Lockwood 1988). Following maturation, part of the energy obtained from food is allocated to reproduction (Lockwood 1988). If food resources are abundant more energy will be used for the production of eggs which might be the case for some of the mackerel near sea cages (Lockwood 1988).

Mackerel needs to continuously swim (lack of swimbladder) which raises the energy requirements of the fish (Juell et al. 1998). In a laboratory setting, Pepin et al. (1988) noted that mackerel did not satiate feeding on zooplankton which indicates food restricted growth (Juell et al. 1998). The readily available prey around the sea cages and the high energy waste pellets can improve the growth of mackerel. Mackerel (1 and 2-year old) held in captivity (8-9 months period) and offered high energy diet (~ 30% fat content) showed rapid increase in condition and lipid content (Juell et al. 1998). Juell et

al. (1998) also noted that for mackerel of the same age the increase in mass was higher than that of length. The authors related this to the availability of food whereas growth in length is restricted by the potential size that the fish can reach. In another experimental study, mackerel held in cages and fed a high energy salmon diet (30% lipid content) for six months doubled in body mass and the lipid content of muscle tissues increased from 19.5% to 30% (Fjermestad et al. 2000). Fjermestad et al. (2000) also reported that the body mass and lipid content increased in mackerel fed a cod diet containing 15% lipids and noted that the gain in mass and lipid was similar to that of the mackerel group fed the salmon diet. Mackerel not offered the artificial feed was filter feeding plankton and maintaining the high lipid content in the tissues; however, when the fish were fed the artificial feed they were noted to be more sedentary and consuming less plankton (Fjermestad et al. 2000). Some mackerel near the sea cages may exhibit similar behaviour.

Mortality of mackerel has also been reported when fed high energy feed (30% lipid content) during the summer months which has been linked to impaired fat catabolism due to the high energy content of the diet (Hamre et al. 1996 cited in Fjermestad et al. 2000). It is unlikely that mackerel feeding off high energy waste pellets near sea cages would lead to high mortality rate because the diet of mackerel is not entirely composed of high energy pellets.

Mackerel readily consumed waste pellets in captivity (Juell et al. 1998) and near sea cages (Chapter 4 and this study) which is also detected in the their FA profiles. Based on the linear mixed effects models for individual FAs significant differences were found in SFAs and 18:2n-6. Similar pattern of decrease in SFAs and 18:2n-6 were noted in muscle tissues of mackerel sampled near the same halibut farm in 2013. The diets fed to the cultured halibut in 2013 and 2014 differed in their FA profiles. The overall n-6 FA levels were similar between the two diets; however the n-3 PUFAs were higher in the halibut diet of 2013. Both halibut diets were richer in fish oil such as 22:6n-3 and an overall higher n-3/n-6 ratio as compared to the salmon diet which had higher levels of vegetable oils such as 18:2n-6.

It is worth noting that as in 2013, only some mackerel were found with waste pellets and not all of them had elevated levels of 18:2n-6 indicating that some individuals may spend longer time around the sea cages or different individual consume variety of prey. Scatter plots revealed some individual mackerel sampled near both fish farms to contain higher levels of 18:2n-6 and 18:3n-3, an overall lower levels of n-3/n-6 and had relatively

high FCI and low HSI. Similar patterns were noted for mackerel sampled near the halibut farm in 2013 (Chapter 4).

Although the linear mixed effects models revealed some differences in the individual FAs of mackerel sampled near and away from sea cages using the LDA clearly separated the groups based on all of the 15 selected FAs. The LDA was able to classify 52.2% of the mackerel sampled near and away from sea cages. Although, using the linear mixed effects model showed no statistically significant differences in 18:3n-3 between mackerel sampled near and away from sea cages using the LDA 18:3n-3 appeared to contribute the most to the separation between the two groups. The LDA also showed clear separation between mackerel sampled near the halibut farm in Loch Melfort and those sampled near the salmon farm in Loch Leven. The difference between mackerel sampled near the halibut and salmon farms is related to the differences in the diets fed for the halibut and the salmon. As noted earlier the salmon diet contained higher levels of the FA 18:2n-6, 18:3n-3, 18:1n-9, and lower n-3/n-6 ratios as compared to the halibut diet for 2014. The main contributing FA for the separation between mackerel sampled near the halibut and salmon farms appears to be 18:3n-3.

The LDA correctly differentiated 50% of the mackerel sampled near the halibut farm and 77% of the mackerel sampled near the salmon farm. It is also worth noting that during the fieldwork at both farms the arrival of new individuals was evident which may lead to non-correctly classified individuals in the LDA.

5.4.2 Impacts of two fish farms (halibut and salmon) on whiting

The presence of both fish farms appear to influence the diet of whiting. This was evident from the stomach content analysis where whiting sampled near both fish farms preyed mainly on invertebrates and waste feed and whiting from reference sites preyed on fish and invertebrates. Two of the 32 whiting sampled in 2013 next to the sea cages of the halibut farm also contained pellets. The data for the stomach content analysis of these fish was not used in Chapter 4 as there were no whiting sampled at a reference site.

Other gadoids such as saithe have been found with pellets in their stomachs when caught near cages (Chapter 4; Carss 1990; Skog et al. 2003). Fernandez-Jover et al. (2011a) reported 6-96% of the diet of cod and saithe near fish farms in Norway was composed of waste feed. In contrast, Mente et al. (2008) studied the diets of demersal fish including whiting at four sea lochs that support fish farms on the West Coast of

Scotland and did not find any pellets in the diet of whiting. The diet of whiting consisted mainly of *Malacostracan crustacea* (e.g. shrimp) and teleost fish (e.g. clupeids and gadoids) (Mente et al. 2008). Dietary difference between lochs were noted but dietary differences related to the presence of fish farming were less consistent with differences found for individual lochs (Mente et al. 2008). Mente et al. (2008) did not find clear causal relationship between fish farming development and impacts on diet composition. Moreover, Mente et al. (2008) noted lack of clear aquaculture influence on the diets of the sampled fish might be related to the sampling methodology which was using bottom trawlers within 50 m from the nearest sea cages. In the present research, sampling took place at the sea cages using rod and line which selects for feeding fish. The presence of waste pellets in whiting sampled next to the cages indicates direct effect of the halibut and salmon farms. Although this may indicate a local-only effect as Mente et al. (2008) pointed out there may be a wider-scale ecological impact of fish farming on marine fish populations.

The abundance of prey reduces foraging times of an animal which results in improved biological condition (Oro et al. 2013). However, no clear differences in length, mass, FCI, HSI and total lipids in muscle tissues were found between whiting samped near and away from sea cages. This may indicate that the fish near and away from cages are feeding on diets of similar energy content. Another explanation for lack of differences in the length, mass, FCI, HSI and muscle lipid content is the high variability in the data which may be related to the age of the fish. Whiting sampled near the halibut farm and both reference sites (1 and 2) were all 0-age group (see Appendix A). Whiting sampled near the salmon farm ranged from 0 to 1 years (see Appendix A).

The scatter plots indicated that some individuals and mainly those sampled near the salmon farm were longer, heavier, had high FCI and HSI. Based on the length at age key these individuals were approximately of age 1. Similar, results were noted for saithe sampled near the halibut farm in 2013. It is worth noting that the HSI of the whiting of the 1-year old individuals sampled near the salmon farm was similar to the HSI of whiting of 2-year old sampled at the Reference site 3 (see Appendix C). This may indicate better food supply for some young whiting near the sea cages with extra energy stored in the livers. In gadoid species, high HSI indicates high total lipid energy which is a direct proxy for egg production (Marshall et al. 1999). The abundance of food and high energy pellets near fish farms might induce earlier maturation and high HSI in some individuals that would lead to higher egg production.

Most whiting mature by the age of 2 (Bailey et al. 2011). It is not clear from this study whether the whiting sampled near the sea cages are sexually mature or not. Further studies are needed to evaluate whether impacts of fish farming might have different effect on male and female fish.

The influence of both the halibut and the salmon farm on the diet of whiting sampled near the cages was evident in their modified FA profiles. Linear mixed effects models indicated statistical differences in a number of individual FAs including 18:3n-3, 18:2n-6, 18:1n-9 and n-3/n-6 ratios between whiting sampled near the two fish farms and reference sites. Similar results were noted for mackerel and saithe (this Chapter and Chapter 4) but results for whiting showed clearer differences between fish sampled near and away from the sea cages. The scatter plots also indicated that some of the whiting sampled near the sea cages had elevated levels of 18:2n-6, 18:3n-3 and 18:1n-9 with lower levels of n-3/n-6 and some of these fish sampled near the salmon farm had high FCI and HSI. As discussed earlier in the disussion this may be age related. Although not included in the analysis the FA profiles of whiting from the third reference were overall similar to the FA profiles of whiting sampled from Reference sites 1 and 2 (see Appendix C).

The FA 20:4n-6 is an important precursor for biologically active compounds such as prostaglandins that play a role in reproduction and also increased levels of dietary 20:4n-6 has been linked to production of more eggs and improved egg and larval quality (reviewed by Bell and Sargent 2003; Salze et al. 2005; Røjbek et al. 2014). The FA 20:4n-6 is also important for growth and development of juvenile marine fish (Bell and Sargent 2003). In the present study, the levels of 20:4n-6 were higher in whiting sampled near the sea cages than those from reference sites which was not found for mackerel and saithe (this study and Chapter 4). This is also in contrast to results reported by Fernandez-Jover et al. (2009). Fernandez-Jover et al. (2009) evaluated the FA profiles of juvenile fish sampled near sea cages and found lower levels of 20:4n-6 as compared to those sampled away from the sea cages. The reason for the higher levels of 20:4n-6 in this study may be because of differences in diets of whiting near and away from cages. Whiting near the sea cages consumed crustaceans (e.g. shrimp, crabs) and those away from the cages consumed fish. Whiting from the Reference site 3 had similar levels of 20:4n-6 to the whiting sampled near the sea cages (see Appendix C) and based on stomach content analysis consumed similar levels of fish and crustaceans (nephrops, crabs) (data not shown). van Deurs et al. (2016) used fatty acid trophic markers to evaluate migrant-

resident interactions and lipid transportation between a local and a distant ecosystem. The researchers reported that the levels of 20:4n-6 were higher in the liver tissues of cod (resident) that have consumed shore crab than herring (migrant).

On the other hand, most marine fish and invertebrates have limited ability to efficiently convert FAs with 18 carbon chains to PUFAs such as arachidonic acid or 20:4n-6 (Arts et al. 2001; Tocher 2003). The higher retention of 20:4n-6 in the tissues of whiting sampled near the sea cages may indicate potential ability of the species to perform such conversions efficiently. Koussoroplis et al. (2011) noted that some fish sampled in a Meditteranean lagoon also retained arachidonic acid or 20:4n-6. The researchers suggested that fishes in estuarine environments may have a different enzyme activity than fish from more open waters. This could be the case in the present study, however, further studies are needed to provide evidence whether this is true regarding the ability of whiting to convert 18:C FAs to PUFAs.

As for mackerel and saithe, not all of the whiting that had elevated levels of terrestrial biomarkers (e.g. 18:2n-6) had waste pellets in their stomach which indicates variability in diets and/or variation in the time spent around the sea cages. In cultured fish such as Atlantic salmon (Bell et al. 2003), cod (Jobling et al. 2008), and European bass (*D. labrax*) (Mourente et al. 2005) when fed on diets with a significant inclusion of vegetable oils for several months levels of 18:2n-6 and 18:3n-3 increased in their tissues. Similarly, Olsen et al. (2015) reported that cod fed diet rich in vegetable oils had elevated levels of FAs (18:2n-6 and 18:3n-3) and cod fed herring diet had elevated levels of marine oil FAs (20:1n-9 and 22:1n-11). Moreover, it was noted that vegetable oils incorporate more slowly (~ 2 months) than marine fish oils (~ 1 month) in fish tissues. Thus, this may indicate that some of the whiting near both the salmon and the halibut farm have spent at least two months to have their FA profiles modified. It is also worth noting that the diet of the halibut farm is richer in marine oils such as 20:1n-9 and 22:1n-11 which may indicate that whiting have stayed at least a month near the halibut farm. Tagging studies are needed to evaluate the residence times and movement patterns of fish near sea cages in the lochs.

The LDA revealed clear separation between whiting sampled near the two fish farms and those sampled from reference sites. The LDA was able to classify 90.4% of whiting sampled near and away from the sea cages. The classification was much higher than that for mackerel (52.2%) indicating a stonger influence of both the halibut and the salmon farms on whiting than on mackerel. The FA 18:2n-6 appears to be a clear

contributor towards the separation between farm and reference sites. The LDA was also able to classify 89.5% of the whiting sampled near the halibut farm and 76.5% of the whiting sampled near the salmon farm. Similar to the LDA results of mackerel, the FA 18:3n-3 appears to be a strong signal for the salmon farm. Fernandez-Jover et al. (2011a) also used LDA to distinguish between cod and saithe sampled near and away from sea cages in Norway. The LDA classified 88.5% and 96.7% of the cod muscle and liver, respectively and 85.7% and 96.7% of the saithe muscle and liver, respectively (Fernandez-Jover et al. 2011a).

The LDA was also able to distinguish between whiting sampled at the two reference sites. The LDA classified 95% of the whiting to Reference 1 and 100% of the whiting to Reference 2. The reason for this difference is possibly because of different diets at the two sites. It is also worth noting that within the whiting sampled at Reference 1 there were two distinct groups. Based on the length at age key for whiting all the fish within this group appear to be 0-age. However, about half of these fish were slightly longer and heavier which may be related to differences in diets.

As noted earlier whiting sampled near the sea cages are likely to be immature fish which is consistent with reports that sea lochs act as nursery grounds for gadoids (Gordon 1981; Bailey et al. 2011). Further studies using bioenergetic modelling approaches might be useful to understand how individual wild fish benefitting from the particulate organic waste from the fish farms would impact the population. Do wild juvenile fish feeding on waste feed mature earlier and how does that impact the population?

5.4.3 Comparison between the halibut and salmon farm

As mentioned in Chapter 1, salmon farming is the dominant fish farming activity in Scotland but interest in halibut farming has been developing (Davies and Slaski 2003). Halibut, reared in salmon sea cages with the addition of a taut tarpaulin, are usually placed in more sheltered waters than salmon sea cages (Davies and Slaski 2003) which was the case for the halibut farm in this study. Both the halibut and salmon are fed with diets that are formulated for each species. The halibut in 2013 and 2014 were towards the end of the production cycle whereas the salmon farm were at the beginning of the production cycle. The stage of production would require different diets. Replacement of vegetable oils in fish diets results in lower levels of PUFAs in fish tissues and to elevate the levels, farmed fish are fed a finishing diet that contains high levels of marine oils (Hixson 2014).

Some of the feed from both types of fish farming will be lost to the environment. More of this waste feed is expected to be lost through salmon cages than the halibut farming. The reason for this is that halibut is a sedentary species and the presence of tarpaulin would allow some of these waste pellets to be consumed by the halibut (Davies and Slaski 2003). Some of the feed will also be indigested by both the halibut and the salmon. The average feed conversion ratios for halibut are 1.3 and for salmon about 1.1-1.2 (Davies and Slaski 2003). The rest of the feed is converted in fish biomass and some is excreted as dissolved nutrients that become available for microbial and primary production (Davies and Slaski 2003). As mentioned earlier there is potential for phytoplankton growth in Loch Melfort but less likely to be the case for Loch Leven because of the shorter flushing time.

The impacts of nutrient wastes on the benthos might be stonger in the halibut farming because they are often placed in more sheltered areas (Davies and Slaski 2003). Based on a model by Davies et al. (2004) the overall wastage from halibut farming is calculated to be much less for an equal biomass production of salmon. However, this may not be the case in this study because the farms were located in different places and harvested different biomasses. Nevertheless, both farms do produce particulate organic matter that is degraded by bacteria which may increase the biomass of bacterivores (e.g. microflagellates) (see Mente et al. 2008 and references therein) which then could be consumed by other trophic levels (e.g. zooplankton).

Although the halibut farm was much smaller in scale as compared to the salmon farm both farms appear to impact mackerel, saithe and whiting sampled near the sea cages (see also Chapter 4). All three species had consumed some of the waste pellets and fatty acid profiles were modified. It was also clear that some individual fish benefit in terms of improved condition. As indicated by results of this chapter and Chapter 4, the FA profiles of the three species are affected with unknown physiological consequences. Both farms used feed that had elevated levels of terrestrial biomarker 18:2n-6 but the salmon farm also had higher levels of 18:3n-3 which was also detected in some of the sampled fish.

The use of individual FAs as biomarkers (e.g. 18:2n-6 and 18:3n-3) of terrestrial origin should be taken with caution as some of these FAs are also present in low levels in the marine environment (Fernandez-Jover et al. 2011b). Using multivariate techniques such as LDA was useful as they are more powerful in finding the patterns in FAs that distinguished among groups.

Other human activities such as sewage disposal and agriculture can increase the input of terrestrial FAs (see Ramírez et al. 2013 and references therein; see also Chapter 2). In the present study, there were some sewage and agricultural discharges in Loch Melfort (Scottish Sanitary Survey Report 2015) and Loch Leven (Scottish Sanitary Survey Reports 2010, 2012). However, it is not expected that these discharges are significant because of low human population in the area and minimal agriculture. Further investigation into FA profiling in the lochs and of different organisms and also near sewage outfalls might be useful.

In general, farmed fish that are fed commercial feed develop, grow and reproduce. However, the increased use of vegetable oils in commercial feeds (see Chapter 2) affects the proportions of polyunsaturated fatty acids (PUFAs) (20:5n-3, 22:6n-3, 20:4n-6) in the farmed fish. These PUFAs are crucial for marine fish reproduction; egg numbers and quality, hatching success etc. (e.g. Bell and Sargent 2003; Salze et al. 2005; Røjbek et al. 2014). Lower levels of PUFAs have also been reported in wild fish sampled near sea cages (e.g. this study and Chapter 4; see also Fernandez-Jover et al. 2011b). Thus, although feeding on high energy feed can contribute towards spawning success the change in FA profiles may offset this. As noted from this and the previous study (Chapter 4) the commercial diets differed between years and the fish species that was cultured. Additionally, all the fish that were sampled consumed a variety of items. Thus, the impact of the change in FA profiles in wild fish consuming the waste feed may not be as strong as that for farmed fish. It is also worth noting that as soon as fish cease feeding on these diets the FAs of terrestrial origin (18:C) are removed or decline progressively from tissues (e.g. Regost et al. 2003; Izquierdo et al. 2005).

Based on results of this and the previous study it is clear that mackerel, saithe and whiting are attracted to the food availability around the sea cages of both farms. However, it is not clear from this and the previous study whether there are other reasons for attraction such as the sea cages themselves or noise created by the farms, or the cultured species themselves (see Chapter 1).

5.4.4 Study limitations

In terms of the study design, the number of replicates at the farm was limited to two which limits the generalization of results across the spatial extent of fish farming in Scotland. The choice of farms was also restricted to the availability of resources which

limits an important aspect of field studies which is randomisation (see Chapter 2). The reference sites for whiting were also not ideal because they were from two distinct regions on the West Coast. Reference sites within the same region as that of the farm of interest would be better for comparison. Using lochs without fish farming activitiy as comparison sites would be quite useful. However, it is difficult to find lochs along the West Coast that do not have any aquaculture activity (Dr. Tom Wilding, SAMS, pers. comm., January 2017). The sampled fish were also restricted to those sampled using rod and line and therefore limits the inference to all wild fish communities. Based on length at age keys, the fish appear to be of different age which is mainly an issue for the mackerel. However, using length as a proxy for age is limited. Thus, further otolith studies would be useful in taking this aspect into account.

Using results from this study and the previous (Chapter 5) can improve future studies related to aquaculture and wild fish interactions. A better sampling design should aim at better control sites, randomization and replication (Hulbert 1984; Underwood 1997).

In terms of the different approaches to the data analysis both the linear mixed effects models and LDA were useful in distinguishing between the different fish groups. Based on fewer assumptions, the LDA appeared to have more power to ordinate the different fish samples based on their fatty acid profiles. The assumptions of the linear mixed effect models were moderately violated (heterogeneity of variances and tailing in the distribution plots) which reduces the power of the models. Although from statistical point of view the assumptions were to some level violated the models provided information that some of the fish sampled near the sea cages are new arrivals that do not exhibit the same changes as the longer resident fish. For example, the diagnostic plots of the FA 18:2n-6 for whiting (Figure C.4) indicate high variability near the sea cages which indicates that some whiting near the sea cages stay longer than others and thus are impacted differently.

Some of the data show extreme values. For example, this was noted in the diagnostic plots of mackerel FAs MUFAs, 18:2n-6, 22:5n-3, total n-6 PUFAs, and 18:3n-3 (Figure C.3). Transformations such as log transformation may appear suitable to reduce high values. However, for the measures in this study, there is a well-established body of evidence (e.g. Fernandez-Jover et al. 2007a) that variation in these values is generally additive and within group variation is generally normal. The seemingly extreme within group variation in this study is likely to come from a mix of individuals within groups,

for example fish that have had long term residence at farm sites mixing with new arrivals. A transformation does not correctly model this type of data and is not appropriate here.

If there were a priori ways of measuring the residence time of fish at a farm, then this could be incorporated as a further explanatory variable into the models and this would probably be suitable for explaning the within farm group variation. Moreover, although overall the group averages vary, a fraction of the fish seem to show no effect at all.

Using linear mixed effects modelling approach is a powerful technique particularly in nested study designs (Zuur et al. 2009). In this study, assigning location as a random effect in the model allowed the estimation of the variance of all site effects rather than a variance for each site effect. As I was interested in an arbitrary sample of all sites rather than the sites themselves this approach was useful. However, the model is limited in its application because of the use of low number of groups (Gelman and Hill 2007). Using this modelling approach with the data of this study increased the chances of making Type II error (failure to reject a false null hypothesis). Using a combination of statistical modelling approaches was useful in reducing the chances of making Type II error.

5.5 Conclusions

Results of this study confirmed results of Chapter 4. Both mackerel and whiting sampled near a halibut and a salmon farm consumed waste feed and this was reflected in their FA profiles. The FA 18:2n-6 was noted as a biomarker for the influence of both the halibut and the salmon farms evaluated in this study. The FA 18:3n-3 was an additional biomarker that could be used to detect the salmon farm influence. Although, no strong evidence was found for improved condition in mackerel and whiting it was clear that some individuals showed improved condition. The overall impact of the two farms was stonger in whiting than in mackerel.

Using a combination of empirical methods was useful in detecting the influence of the two fish farms on wild fish. Additionally, using a combination of univariate and multivariate modelling approaches was also useful in analysing the data. The use of multivariate modelling approach was a more powerful technique in detecting influence of the two fish farms on the sampled fish.

CHAPTER 6
EVALUATING THE POTENTIAL OF TWO COASTAL FISH FARMS TO ACT AS ECOLOGICAL TRAPS OR PRODUCTIVITY SITES

6.1 Introduction

Coastal fish farms attract high densities of various pelagic to benthic fish species (see Chapter 1 for review; Appendix A). The increase in fish abundance can be a result of fish moving from the surrounding area towards the farm with no overall increase in local production (attraction hypothesis). Fish can also settle, grow, reproduce and consequently contribute to the production of the population (production hypothesis) (Bohnsack 1989; Lindberg 1997; Pickering and Whitmarsh 1997). Reubens et al. (2014) added that artificial habitats may also act as ecological traps leading to an overall reduced fitness of the population. Ecological traps, often caused by anthropogenic activities, are situations in which animals actively select to settle in habitats that are poor for survival and reproduction over better habitats (Dwernychuk and Boag 1972; Battin 2004; Robertson and Hutto 2006). Ecological traps affect a variety of taxa (e.g. birds, arthropods, mammals, reptiles, amphibians, fish) however majority of the research on traps were found for birds (reviewed by Hale and Swearer 2016).

The majority of studies related to ecological traps have been conducted in terrestrial systems (reviewed by Battin 2004; Robertson and Hutto 2006; Hale and Swearer 2016) and few studies have addressed ecological traps in aquatic systems (Hallier and Gaertner 2008; Dempster et al. 2011; Reubens et al. 2013; Gutzler et al. 2015). In a study by Hallier and Gaertner (2008), tuna associated with fish aggregating devices (FADs) were found in poorer condition than those in free schools. This was related to reduced food availability for fish associated with the FADs resulting in high competition (Hallier and Gaertner 2008). Thus, fish aggregating devices can act as ecological traps by misleading tunas resulting in potentially increased mortality rates and disruption to migratory routes (Hallier and Gaertner 2008).

Dempster et al. (2011) evaluated whether fish farms act as ecological traps or population sources for cod (*Gadus morhua*) and (*Pollachius virens*) associated with fish farms. As the overall fish farming impacts were positive on the wild fish the authors concluded that fish farms act as population sources. Populations are at increased extinction risk when trapped in unsuitable habitats. Thus, gaining knowledge on how ecological traps are created and how the behaviour of animals that actively choose them is affected can reduce the risk of population extinctions (Schlaepfer et al. 2010).

Different fish species respond differently to the presence of artificial habitats (see Chapters 4 and 5). In this chapter, proxies of fitness such as condition and diet of mackerel, whiting and saithe were used to assess whether sea cages can be potential ecological traps or population sources.

6.2 Methods

In this chapter, I combined data related to the size, condition and diet for each species (mackerel, whiting and saithe) sampled near two fish farms during the fieldwork of 2013 and 2014 (Chapter 4 and 5). The number of individuals of each species per year and location for which data is available for length, mass, condition indices (HSI and FCI) and diet can be found in Table 6.1.

Table 6.1 Number of individual mackerel, whiting and saithe sampled during summers of 2013 and 2014 near and away from sea cages.

	Mackerel		**Whiting**		**Saithe**	
Locations	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
Leven Farm	0	17	0	54	0	3
Melfort Farm	28	110	31	41	7	6
Reference Mackerel	22	0	0	0	0	0
Reference Mackerel 1	0	69	0	0	0	0
Reference Mackerel 2	0	67	0	0	0	0
Reference Mackerel 3	0	45	0	0	0	0
Reference Whiting 1	0	0	0	40	0	0
Reference Whiting 2	0	0	0	55	0	0
Reference Saithe	0	0	0	0	7	0

6.2.1 Data analysis

All the data was analysed using the statistical software R (R Development Core Team 2016) run in RStudio (version 1.0.136, RStudio Team 2016). As in the previous two chapters prior to applying any statistical models to the data graphical exploratory

tools (boxplots and Q-Q plots) were used as suggested by Zuur et al. (2010) (for more detail see Chapter 4).

To obtain an idea about community structure of fish sampled near the two fish farms length-frequency distributions were built for each species. Fulton's condition index and hepatosomatic index (HSI) were calculated to give an overall indication of the general condition for all individuals of the three species sampled near the two fish farms and those from reference sites. Total mass (somatic mass plus gonads and stomach contents) was used in this study as somatic mass was unavailable. Total length (cm) was used for the analysis of whiting and saithe. Fork length (cm) was used for the analysis of mackerel because during sampling of 2013 the tails at the edges were partially lost during storage.

Linear mixed effects models were used to evaluate whether there were differences in length, mass, FCI, HSI between mackerel and whiting sampled near and away from sea cages. To account for size-correlated variation, length of fish was included in the models as an independent variable, and dropped if found not significant. Year was included as a random factor in the models with multiple years of data (mackerel only). The factor year was found insignificant because of the low sampling sizes and therefore it was dropped from the models for mackerel. Year was not included in models for whiting because of insufficient sampling sizes to compare between years. The parameters in the models were estimated using the maximum likelihood method. All linear mixed effects models were built using the packages lme4 (Bates et al. 2015). The package lme4 does not provide p values for the fixed effect in the models and therefore the package lmerTest (Kuznetsova et al. 2016) was used to approximate the p values.

An ANOVA model was used to test for differences in length and mass of saithe sampled near and away from sea cages and an ANCOVA model was used to test for the differences in FCI and HSI of saithe near and away from sea cages. The reason for using an ANOVA/ANCOVA models rather than linear mixed effects models was because the sample sizes and number of locations was not enough to fit the model. All model assumptions (normal distribution, homogeneity of variance) were evaluated by visually inspecting the residuals and fitted values.

Length-mass relationships were used as an index of well being to compare the condition of mackerel, whiting and saithe sampled near the two fish farms and at reference sites. The length-mass relationships were calculated using equation 2.1 (see Chapter 2; Froese 2006). To calculate the coefficients (*a* and *b*) of equation 2.1, linear

regressions were fitted by the least square method following log transformation of the variables W and L:

$$logW = loga + blogL \qquad (eq.6.1)$$

Linear mixed effects models were fitted to the length-mass relationships for mackerel and whiting. Linear regressions were fitted to the mass-length relationships of saithe sampled near and away from sea cages.

To get a general overview of the dietary composition of the three species the frequency of occurrence (FO (%)) of diet items were summarised for the years 2013 and 2014. Multiple correspondence analysis (MCA) is a multivariate technique used in the analysis of categorical data. MCA was used to explore the patterns of diet of mackerel, whiting and saithe sampled near fish farms and references sites. The analysis was run using the package FactoMineR (Le et al. 2008) using function MCA in the software R. The packages ggplot2 (Wickham 2009) and cowplot (Wilke 2015) were used to plot the data.

6.3 Results

6.3.1 Sizes and condition of fish near and away from cages

Length frequency distributions for all three species can be found in Figures 6.1-6.3. Summaries for model outputs for length, mass, FCI and HSI for mackerel, whiting and saithe can be found in Tables 6.2, 6.3 and 6.4, respectively. FCI and HSI plots can be found in Figures 6.5 and 6.6, respectively. Diagnostic plots for all models can be found in Appendix D.

For the summers of 2013 and 2014, a total of 155 and 181 mackerel were sampled near two fish farms and at reference sites, respectively (Table 6.1). Length-frequency distribution for mackerel can be found in Figure 6.1. The length of mackerel sampled near the sea cages ranged from 15.8 cm to 38.1 cm which based on length at age key (see Appendix A) corresponds to ages between 0 and 11. The length of mackerel sampled near the two fish farms were statistically significantly different (28.4 cm, 95% CI: [27.3, 29.2]) than those caught at reference sites (24.0 cm, 95% CI: [23.5, 24.4]) (Farm vs Reference difference, 5.0, 95% CI: [1.52, 8.50], t = 3.55, p = 0.01) (Table 6.2). The mass of mackerel sampled near the sea cages was statistically significantly different (278 g, 95% CI: [255.6,

302.6]) than the mass of mackerel sampled from reference locations (146 g, 95% CI: [138.1, 153.9]) (Farm vs Reference difference: 148.7, 95% CI: [84.8, 213], t = 6.29, p = 0.003) (Table 6.2).

FCI of mackerel caught near the cages ranged from 0.88-1.42 and FCI caught away from cages ranged from 0.72-1.30 (Figure 6.5). Statistically significant differences were noted between both groups of mackerel (Farm vs Reference difference: 0.1; 95% CI: [0.02, 0.19], t = 3.07, p = 0.02) (Table 6.2). HSI of mackerel sampled near the sea cages ranged from 0.84-4.73% and HSI of mackerel away from cages ranged from 0.72-4.73% (Figure 6.6). No statistically significant differences were detected between both groups of mackerel (Farm vs Reference difference: -0.2; 95% CI: [-1.42, 1.07], t = -0.34, p = 0.70) (Table 6.2). The effect of the farm on the length, mass, FCI of mackerel sampled near the sea cages appears to be stonger than the natural variability among sites and years (Table 6.2).

The diagnostic plots for the linear mixed effects models for length, mass and FCI indicated moderate levels of heterogeneity of variances (Figure D.1). As in Chapters 4 and 5, no outliers were removed and no other models were applied because the models provided sufficient information to indicate that only a proportion of the fish sampled near the sea cages are impacted. See also Chapters 4 and 5 for further discussions.

A total of 126 whiting were sampled near two fish farms and 95 at reference locations (Table 6.1). Figure 6.2 contains the length-frequency distributions for whiting sampled near the two fish farms and those sampled at reference sites. The length of whiting sampled near the sea cages ranged from 9.2 to 23.2 cm and whiting sampled away from cages ranged from 10.5 to 20.3 cm. Based on length at age key (Appendix A) both whiting sampled near and away from sea cages ranged from 0 to 1 year. Whiting sampled near both fish farms were similar in length (15.6 cm, 95% CI: [15.0, 16.3]) (Farm vs Reference difference: 1.3, 95% CI: [-3.88, 6.5], t = 0.7, p = 0.522) and mass (37 g, 95% CI: [32.2, 41.0]) (Farm vs Reference difference: 13.1, 95% CI: [-22.4, 48.6], t = 1.02, p = 0.364) to the length (14.4 cm, 95% CI: [14.0, 14.9]) and mass (24 g, 95% CI: [21.6, 27.2]) of whiting sampled at reference sites (Table 6.3).

FCI of whiting ranged from 0.64 to 1.03 near cages and 0.59 to 0.90 away from cages (Figure 6.5). No statistically significant differences were noted in FCI between both groups (Farm vs Reference difference: 0.1, 95% CI: [-0.007, 0.148], t = 2.55, p = 0.065) (Table 6.3). HSI of whiting sampled near the cages ranged from 0.26 to12.02% and HSI of whiting sampled away from cages ranged from 0.30 to 5.04% (Figure 6.6). No

statistically significant differences in HSI were noted between both groups (Farm vs Reference difference: 1.5; 95% CI: [-2.23, 5.29], t = 1.13, p = 0.322) (Table 6.3). There appears to be high variability in length, mass and HSI of whiting amongst the sites (Table 6.3).

The diagnostic plots for the linear mixed effects models for length, mass, FCI and HSI of whiting indicated moderate heterogeneity of variances (Figure D.2). As with the mackerel data, the models were kept because they provided sufficient information on the impact of the two fish farms on the sampled fish near the sea cages.

For saithe the sampling number was low at both farms and the reference site. 19 saithe were sampled near the sea cages and 7 were sampled at a reference site (Table 6.1). Two of the fish sampled near Loch Leven were of much bigger length and mass as compared to the rest of the fish and therefore were removed from the analysis.

The length frequency-distributions for saithe sampled near the sea cages and at references sites can be found in Figure 6.3. The length of saithe sampled near the sea cages ranged from 11.8 cm to 19.1 cm (not including the two bigger fish) whereas the length of saithe sampled away from cages ranged from 17.1 cm to 22.0 cm (Figure 6.3). Based on the length at age key (Appendix A) saithe sampled near and away from sea cages were of 0 age. There were statistical differences in length (Reference vs Farm difference, 4.07, 95% CI: [2.40, 5.73]) (F = 25.55, p = 0.000) and mass (Reference vs Farm difference, 21.93, 95% CI: [8.69, 35.16]) (F = 11.81, p = 0.002) of saithe sampled near two fish farms and the length and mass of saithe caught at a reference site (Table 6.4).

FCI of saithe caught near the sea cages ranged from 0.80 to 1.19 and FCI of saithe caught away from cages ranged from 0.69 to 1.19 (Figure 6.5). Statistically significant differences in FCI were found between saithe sampled near and away from the sea cages (Reference vs Farm difference, -0.18, 95% CI: [-0.27, -0.09], F = 19.06, p = 0.000) (Table 6.4). HSI of saithe caught near the cages ranged from 1.44 to 8.71% and HSI of saithe away from cages ranged from 2.32 to 5.99% (Figure 6.6). No statistical differences in HSI were found between saithe sampled near and away from sea cages (Reference vs Farm difference, -0.24, 95% CI: [-1.54, 1.06], F = 0.15, p = 0.703) (Table 6.4).

The diagnostic plots for the saithe data indicated overall satisfactory model assumptions (Figure D.3).

Figure 6.1 Length frequency distributions of mackerel sampled near two fish farms and at reference sites.

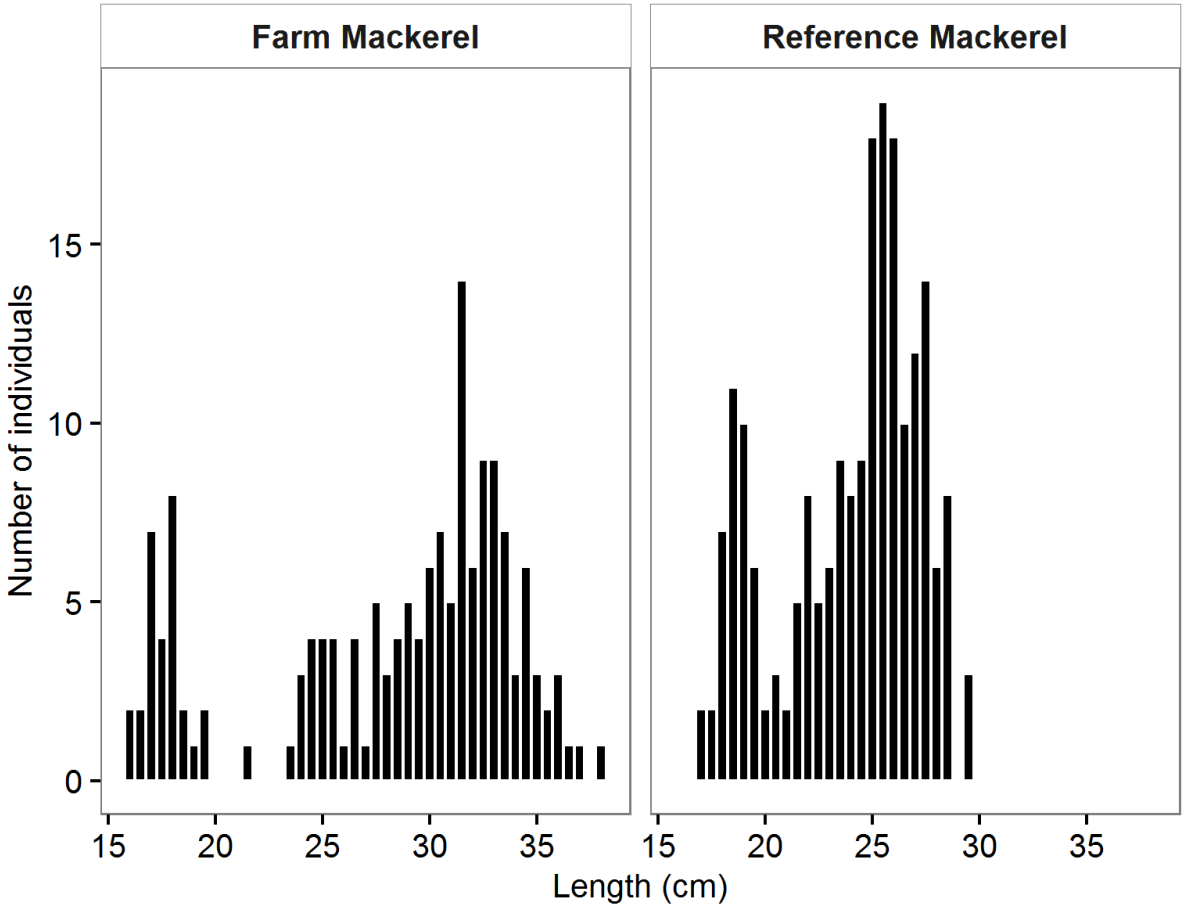

Figure 6.2 Length frequency distributions of whiting sampled near two fish farms and at reference sites.

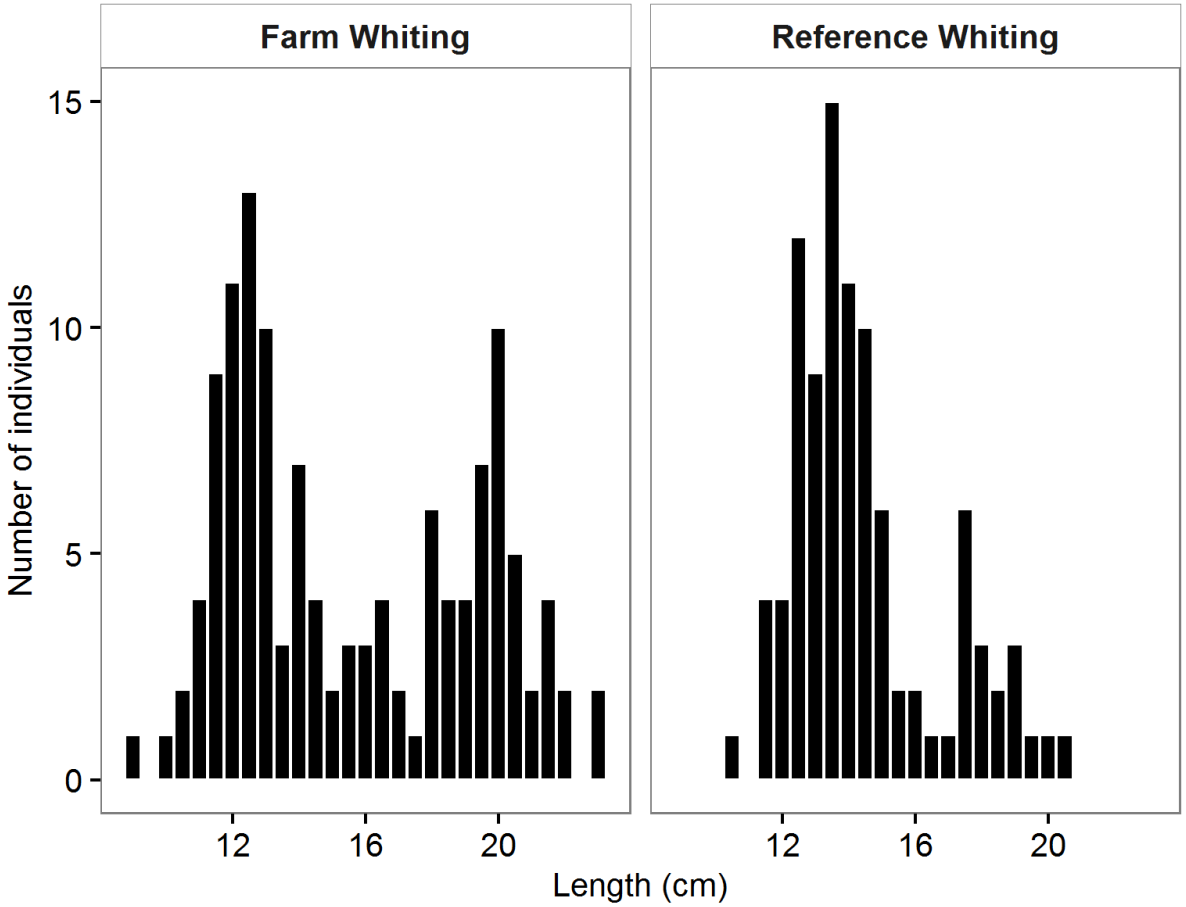

Figure 6.3 Length frequency distributions of saithe sampled near sea cages and at a reference site.

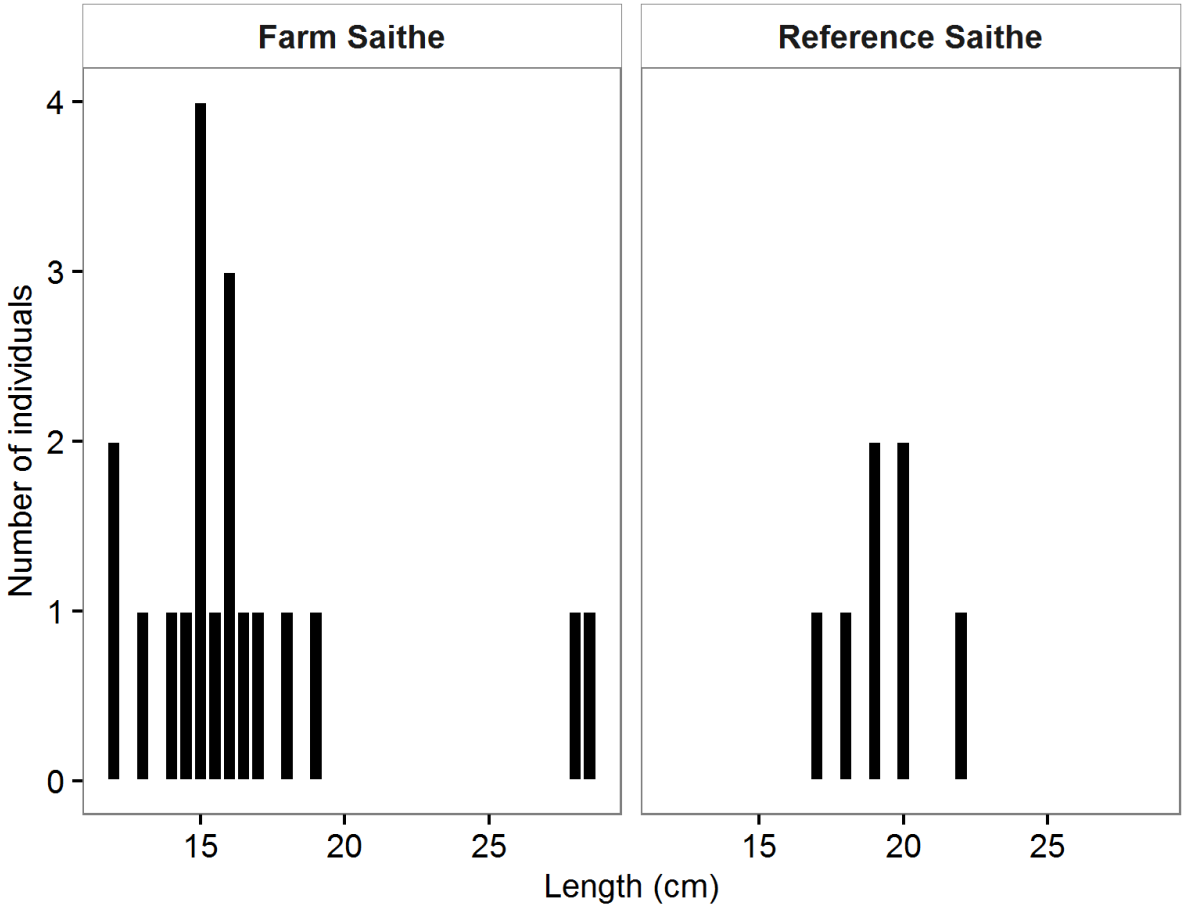

Table 6.2 Linear mixed effects model summary for length (cm), mass (g), FCI and HSI of mackerel sampled near and away from sea cages.
Note: SE: standard error, df: degrees of freedom, Significance level: P < 0.05, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Length (cm)	Intercept	28.980	1.160	5.807	24.991	0.000	Intercept (Location)	2.139	1.463
	Treatment	-5.007	1.411	5.755	-3.548	0.034	Residual	19.365	4.401
Mass (g)	Intercept	293.411	19.410	4.162	15.116	0.000	Intercept (Location)	486.4	22.05
	Treatment	-148.694	23.648	4.294	-6.288	0.003	Residual	10918.7	104.49
FCI	Intercept	1.091	0.028	6.333	39.035	0.000	Intercept (Location)	0.001	0.036
	Treatment	-0.104	0.034	6.253	-3.065	0.021	Residual	0.009	0.094
HSI	Intercept	1.985	0.419	6.174	4.738	0.003	Intercept (Location)	0.387	0.622
	Treatment	0.176	0.512	6.138	0.344	0.742	Residual	0.410	0.641

Table 6.3 Linear mixed effect models output for length (cm), mass (g), FCI and HSI of whiting sampled near and away from sea cages. ***Note:*** SE: standard error, df: degrees of freedom, Significance level: $P < 0.05$, SD: standard deviation.

Variable	**Fixed-effects**						**Random-effects**		
		Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Length (cm)	Intercept	15.944	1.321	3.981	12.07	0.000	Intercept (Location)	3.391	1.842
	Treatment	-1.310	1.872	4.018	-0.70	0.522	Residual	6.075	2.465
Mass (g)	Intercept	38.826	9.037	3.983	4.296	0.013	Intercept (Location)	159.0	12.61
	Treatment	-13.090	12.809	4.018	-1.022	0.364	Residual	266.7	16.33
FCI	Intercept	0.820	0.019	3.738	42.379	0.000	Intercept (Location)	0.001	0.026
	Treatment	-0.071	0.028	3.867	-2.555	0.065	Residual	0.005	0.070
HSI	Intercept	3.424	0.957	3.995	3.578	0.023	Intercept (Location)	1.777	1.333
	Treatment	-1.530	1.354	4.011	-1.130	0.312	Residual	2.797	1.672

Table 6.4 Output of the ANOVA/ANCOVA models used for length (cm), mass (g), FCI and HSI of saithe sampled near and away from sea cages. ***Note:*** Df: degrees of freedom, Sum Sq: sum of squares, Mean Sq: mean sum of squares, Significance level: $P < 0.05$.

Saithe					
Length					
	Df	Sum Sq	Mean Sq	F value	P (> F)
Treatment	1	81.99	81.99	25.55	0.000
Residuals	22	70.61	3.21		
Mass					
	Df	Sum Sq	Mean Sq	F value	P (>F)
Treatment	1	2384	2383.9	11.81	0.002
Residuals	22	4442	201.9		
FCI					
	Df	Sum Sq	Mean Sq	F value	P (>F)
Length	1	0.03	0.03	3.60	0.023
Treatment	1	0.16	0.16	19.06	0.000
Residuals	21	0.18	0.01		
HSI					
	Df	Sum Sq	Mean Sq	F value	P (>F)
Length	1	40.67	40.67	22.24	0.000
Treatment	1	0.27	0.27	0.15	0.703
Residuals	19	34.75	1.83		

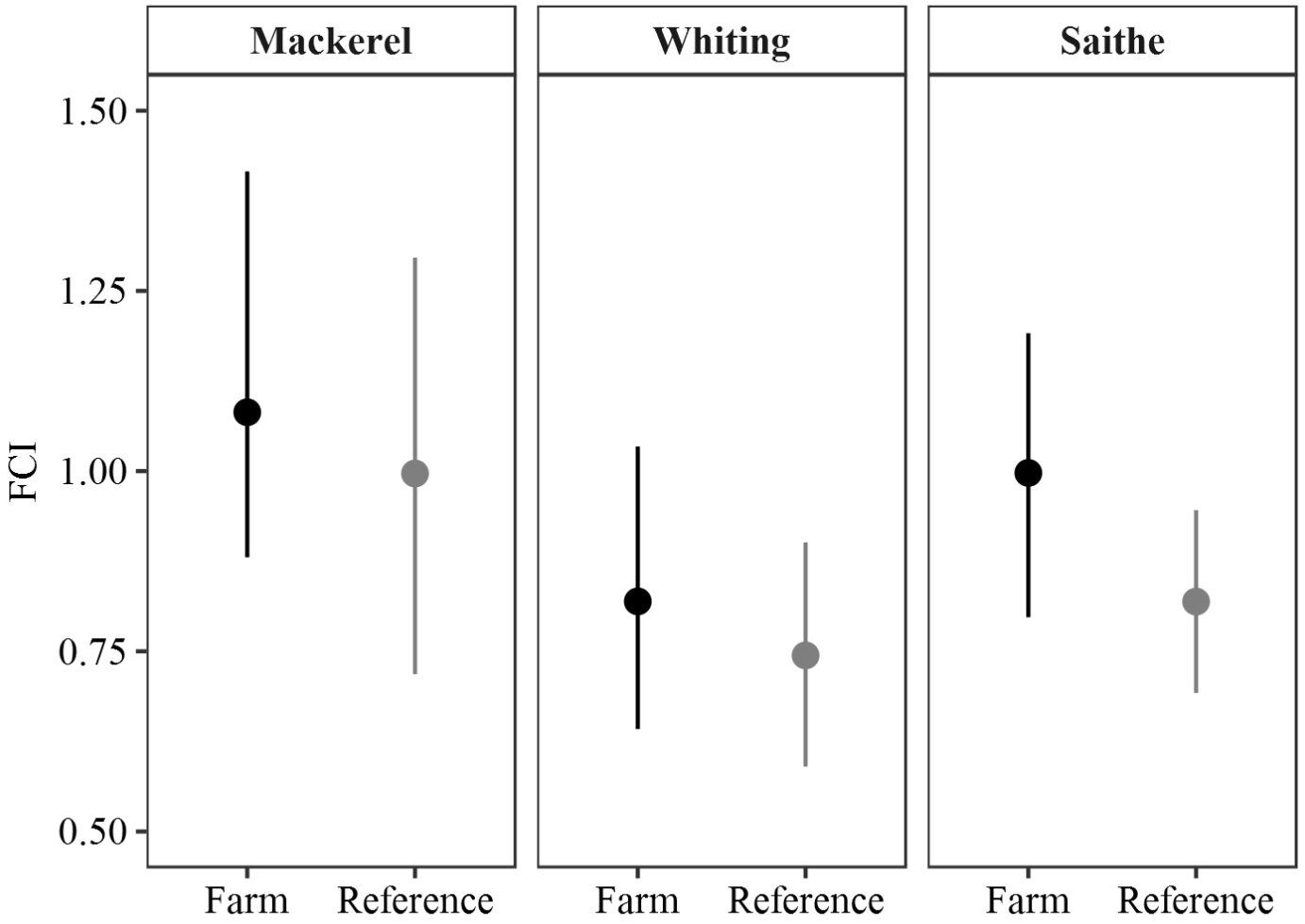

Figure 6.4 Fulton's condition index (FCI) of mackerel, whiting and saithe caught near and away from sea cages.

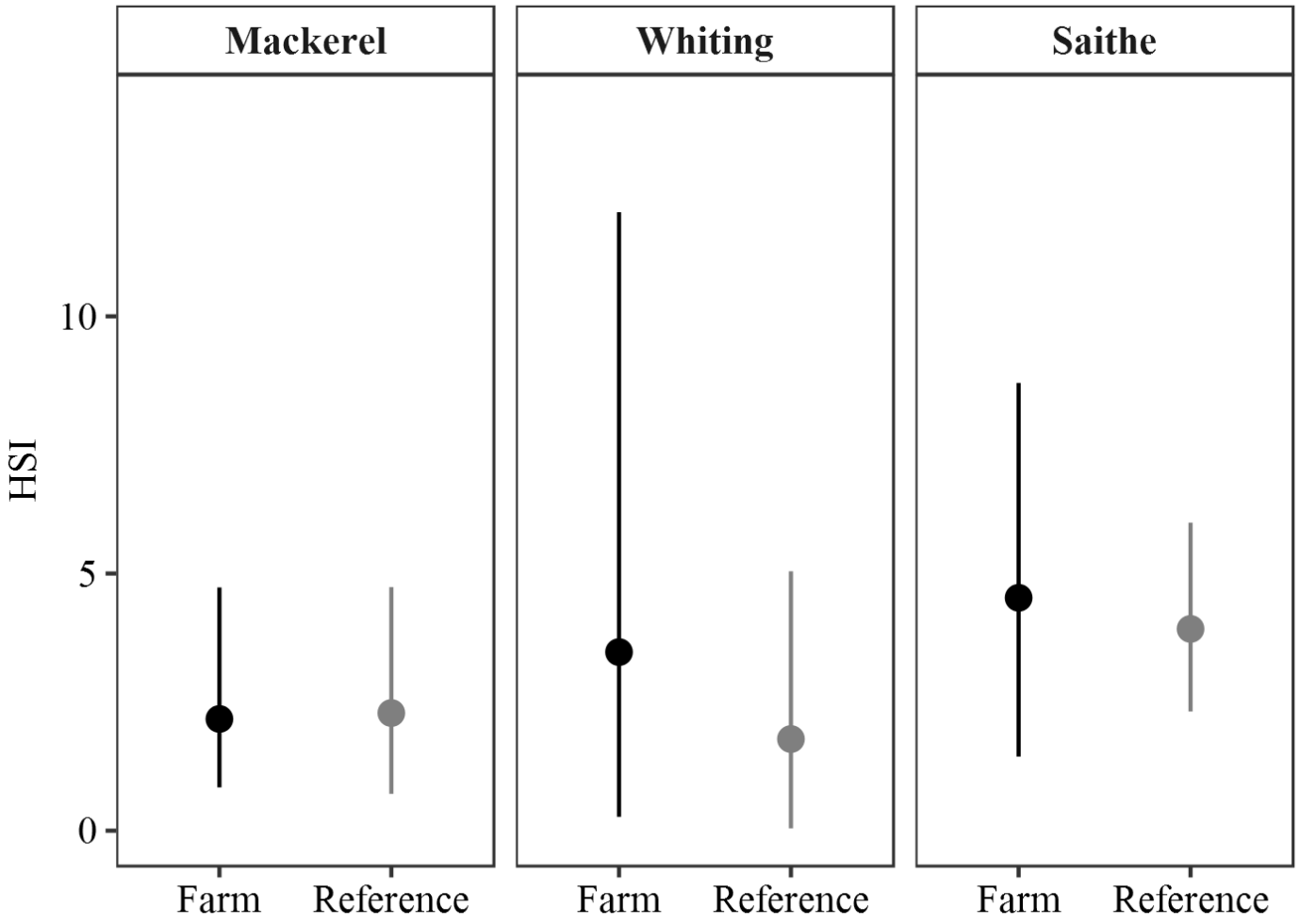

Figure 6.5 Hepatosomatic index (HSI) of mackerel, whiting, and saithe caught near two fish farms and reference sites.

6.3.2 Length and mass relationships

Mass-length relationships of mackerel, whiting and saithe near and away from cages are presented in Figure 6.6, 6.7 and 6.8A,B, respectively. Diagnostic plots for the models can be found in Appendix D.

No statistically significant differences were found between the slopes for the length-mass relationships for mackerel sampled near and away from sea cages (Table 6.5; Figure 6.6). The parameters of the length-mass relationship were found from the coefficients of the linear mixed effects model. The parameters for the length-mass relationships of mackerel sampled near the sea cages were: a = 0.0052 and b = 3.22 whereas those for mackerel sampled away from sea cages were: a = 0.0053 and b = 3.22. The growth of mackerel appears to be allometric (b > 3) (Froese 2006).

No statistically significant differences were found between the slopes of the length-mass relationships for whiting sampled near and away from sea cages when taking into account the variability between sites (Table 6.5; Figure 6.7). The parameters of the length-mass relationship for whiting sampled near both fish farms were: a = 0.0081 and b = 3.00. The parameters for the the length-mass relationships of whiting samples away from the sea cages were: a = 0.0080 and b = 3.00. The growth of whiting appears to be isometric (b = 3) (Froese 2006).

Statistically significant differences were found between the slopes of the length-mass relationships of saithe sampled near and away from sea cages ($p < 0.000$) (Table 6.5). The parameters of the length-mass relationship for saithe sampled near the sea cages were: a = exp(-6.04) or 0.0024 and b = 3.53 with a coefficient of determination (r^2) of 0.970. The parameters for the length-mass relationships of saithe sampled away from the sea cages were: a = exp(-3.32) or 0.0362, b = 2.5 and $r^2 = 0.84$ (Table 6.6 and Figure 6.8A,B).

Table 6.5 Linear mixed effects model output for length-mass data for mackerel and whiting sampled near and away from sea cages. ***Note:*** SE: standard error, df: degrees of freedom, Significance level: $P < 0.05$, SD: standard deviation.

Mackerel								
Fixed-effects						**Random effects**		
	Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Intercept	-5.248	0.085	185.2	-61.908	0.000	Intercept (Location)	0.002	0.042
Log (Length)	3.217	0.024	356.6	136.7	0.000	Intercept (Year)	0.000	0.000
Farm vs Reference	-0.062	0.038	0.038	-1.618	0.154	Residual	0.006	0.080
Whiting								
Fixed-effects						**Random effects**		
	Estimate	SE	df	t-value	P (>|t|)		Variance	SD
Intercept	-4.186	0.108	69.89	-44.403	0.000	Intercept (Location)	0.003	0.052
Log (Length)	3.00	0.073	209.50	80.880	0.000	Residual (Year)	0.008	0.089
Farm vs Reference	-0.09	0.053	1.93	-1.702	0.235			

Table 6.6 Estimates for the linear regression model fit to length-mass data for saithe sampled near and way from sea cages. ***Note:*** SE: standard error, df: degrees of freedom, Significance level: $P < 0.05$.

	Estimate	SE	t-value	P (>|t|)
Intercept	-5.679	0.476	-11.935	0.000
Log (Length)	3.393	0.175	19.410	0.000
Farm vs Reference	-0.292	0.060	-4.889	0.000

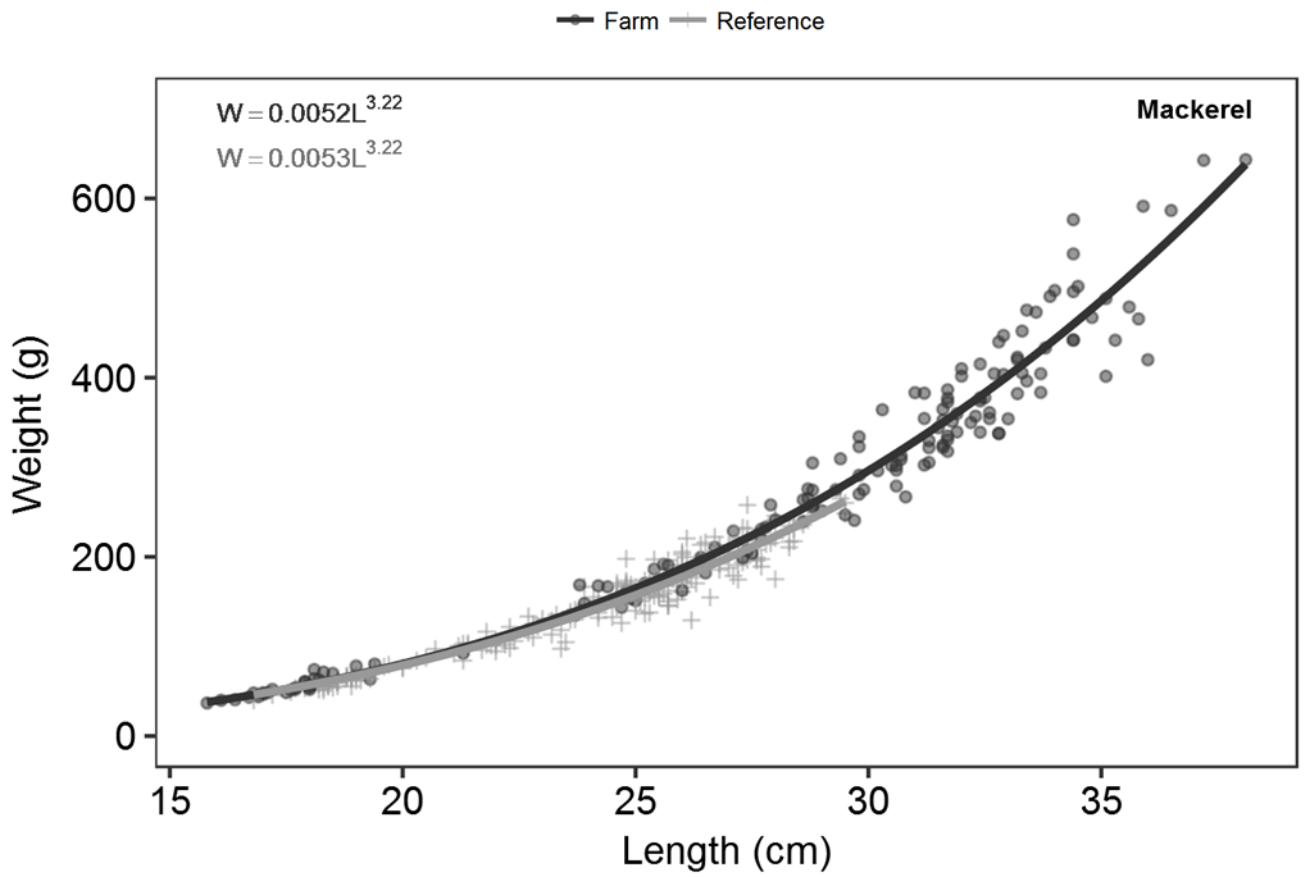

Figure 6.6 Length-mass relationships for the sampled mackerel near and away from sea cages.

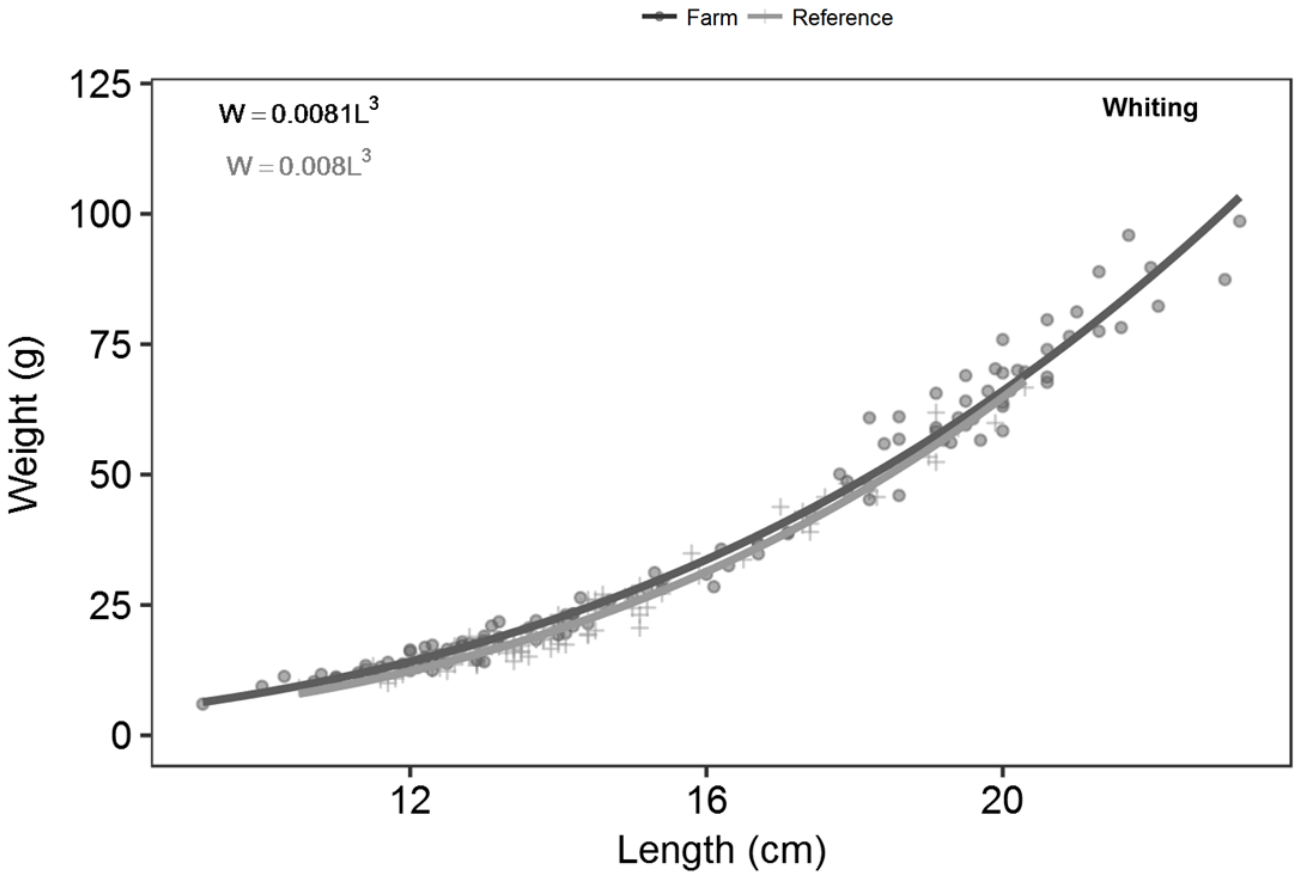

Figure 6.7 Length-mass relationships for the sampled whiting near and away from sea cages.

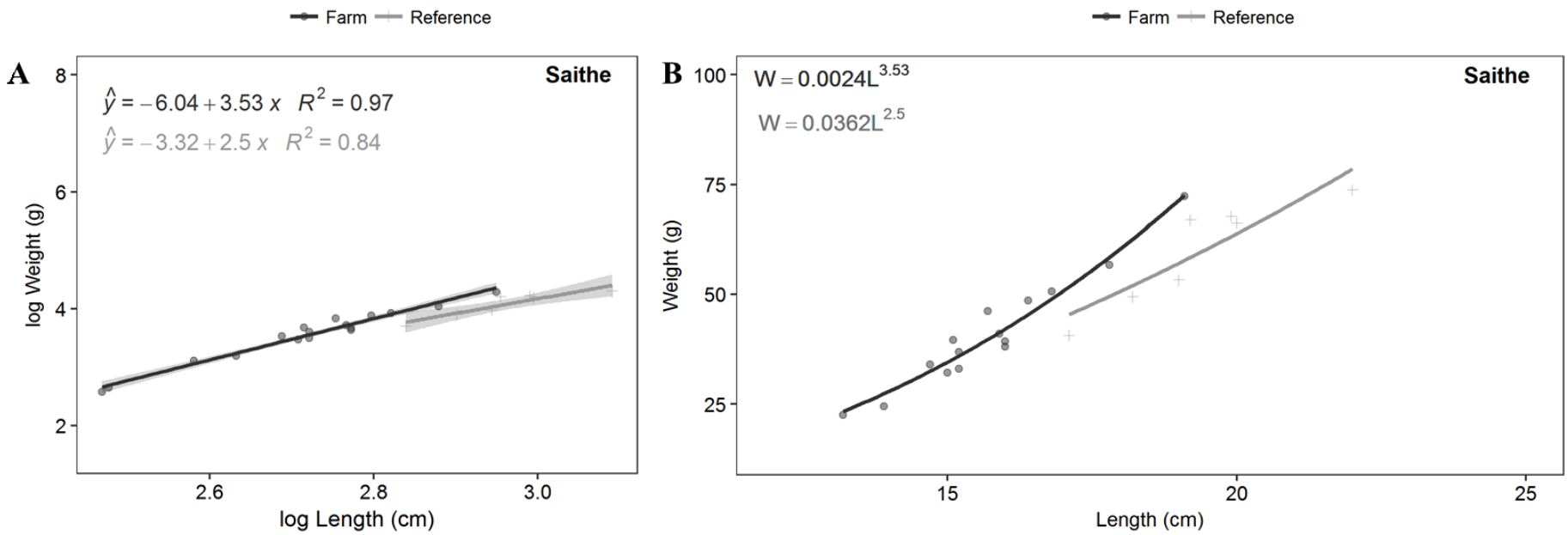

Figure 6.8 Logarithmic mass-length relationships (with 95% confidence intervals) with regression equations (A) and length-mass relationship (B) for saithe sampled near and away from sea cages.

6.3.3 Diets of fish near and away from cages

Summary of all items found in stomachs of mackerel, whiting and saithe sampled near and away from sea cages for both years can be found in Table 6.7 and Figure 6.9.

Fish were the main item for both mackerel near and away from sea cages (Table 6.7). Fish pellets were found in 14.8% of all the mackerel sampled near sea cages in 2013 and 2014. About 25.8% of the mackerel sampled away from the sea cages had empty stomachs (Table 6.7). Invertebrates followed by artificial pellets were found in 74.6% and 34.5% of the whiting sampled near the sea cages (Table 6.7). Invertebrates followed by fish were found in 61.5% and 47.6% of the whiting sampled away from the sea cages (Table 6.7). About 37.6% of the whiting sampled away from sea cages had empty stomachs and 20.5% of the whiting sampled near sea cages had empty stomachs (Table 6.7). Fish were found in majority of the saithe sampled away from sea cages (Table 6.7). Invertebrates followed by fish and artificial feed were found in majority of the saithe sampled near the sea cages (Table 6.7).

Table 6.7 Frequency of occurrence (%) of items found in stomachs of mackerel, whiting and saithe sampled near two fish farms and at reference sites.

	Farm	**Reference Mackerel**	**Farm**	**Reference Whiting**	**Farm**	**Reference Saithe**
Fish	83.1	91.3	7.5	47.6	75	66.7
Fish Pellets	14.8	0	34.4	0	75	0
Invertebrates	1.4	4.9	74.6	61.5	100	66.7
Unidentified	10.6	39.4	24.7	10.3	25	16.7
Empty	8.4	25.8	20.5	37.6	0	50

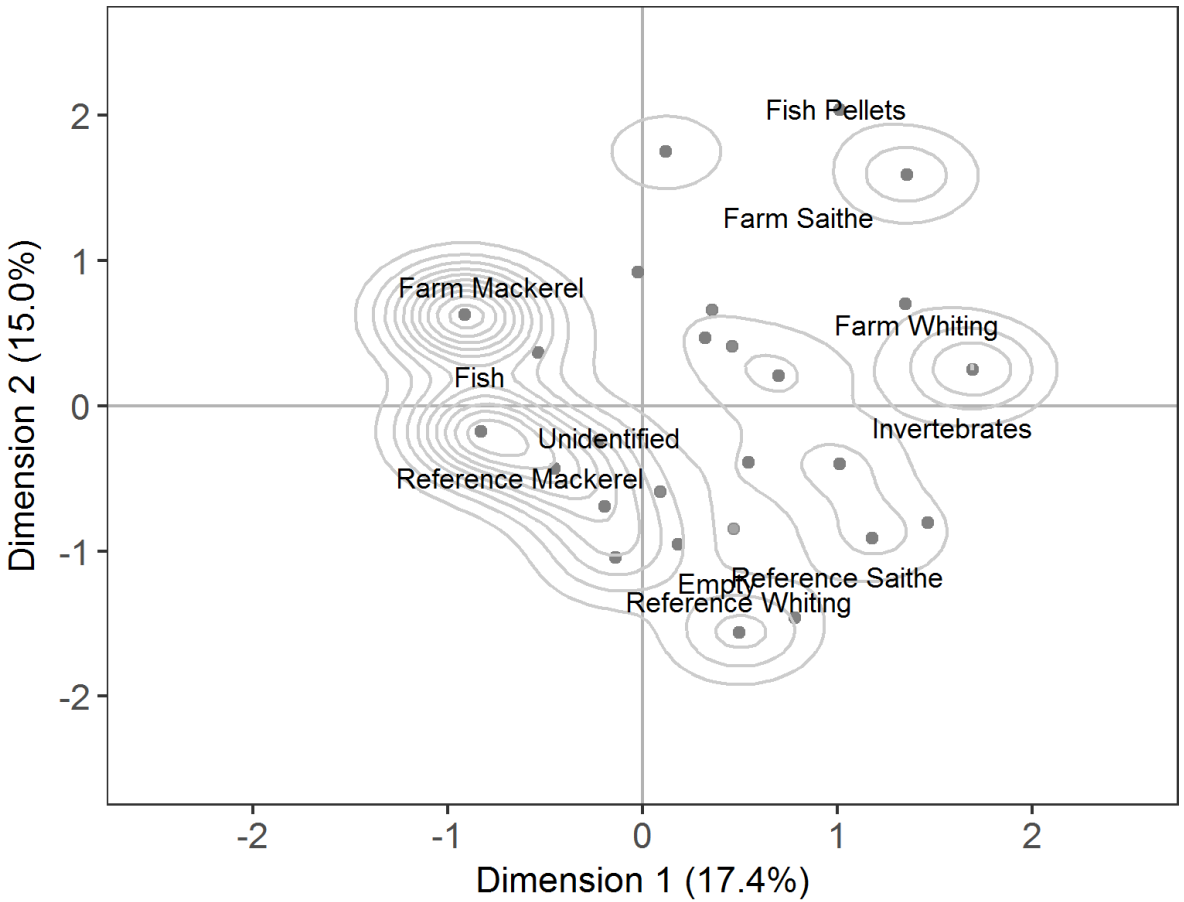

Figure 6.9 Correspondence analysis and density curves (grey dots are individuals that are overlapping) by stomach content items and locations (farm versus reference) for mackerel, whiting and saithe sampled near and away from sea cages.

6.4 Discussion

Fish of different sizes were attracted to the studied fish farms. For mackerel various sizes dominated the catches which indicates different ages. This is consistent with the behaviour of mackerel where, following spawning, fish of all age groups migrate to feeding grounds such as the West coast of Scotland (Lockwood 1988). For whiting and saithe, based on the length at age keys the age groups 0 and 1 are dominating which is consistent with observations that gadoid species use sea lochs as nursery areas (Ware 2009).

It is also worth noting that the patterns of length distribution for both mackerel and gadoid species can be biased by the fish sampling methodology. In this study, mackerel, whiting and saithe sampled near the two fish farms were caught using baited rod and line which is a selective fishing technique. Factors that influence the selectivity of the hook and line technique include type and size of baits, hook design, feeding strategy, and fish ecology (Løkkeborg and Bjordal 1992). Based on anecdotal accounts juvenile flatfish were dominating the catches near the halibut farm on some occasions when the fishing gear was changed. Skate was also sampled when the fishing gear was changed to approximately 25-50 kg rod and 12/0 hook size and 1 kg lead (see Appendix A). Further studies might be useful in exploring the different fish communities around sea cages using various sampling techniques.

In general, the presence of easily accessible and abundant food resource in the environment can improve the body condition and reproductive output of animals that take advantage of such resources (Oro et al. 2013). As noted from the fieldwork studies in 2013 and 2014, there was an abundance of food (e.g. fish, invertebrates, waste pellets) near the two studied fish farms. This was indicated by the lower number of empty stomachs found for mackerel, whiting and saithe sampled near the sea cages as compared to their counterparts. Evidence for this was also found in improved FCI for both mackerel and saithe sampled near the sea cages of both fish farms. It is worth noting that no differences in FCI were detected in Chapters 4 and 5 for mackerel sampled near and away from sea cages. This appears to be related to the sampling size.

HSI is a better index for energy storage in gadoids and improved HSI was noted for some saithe and as indicated in the previous study (Chapter 5) for some individual whiting. Although abundance of resources near the sea cages benefits some fish the food quality is also important. As indicated by the previous two studies (Chapters 4 and 5)

poor food quality can result from low levels of PUFAs which can translate in poor reproductive output (Salze et al. 2005). It is not clear what the long term effects of changes in the fatty acid profiles have on the physiology of fish. Oro et al. (2013) noted that some anthropogenic wastes (e.g. dumps, fish discards) is of poor quality and also linked with contaminants and/or pathogens. The consumption of such food can increase the presence of individuals that are of poor condition or obese and limit their ability to escape predators and also reduce reproductive output (Oro et al. 2013). There was no evidence to indicate that fish sampled near the sea cages were of poor condition.

Based on the length-mass relationship saithe sampled near the sea cages appear to show positive allometric growth ($b > 3$) which indicates that saithe become deeper-bodied with increase in length as compared to saithe away from cages ($b < 3$). The allometric growth for saithe near sea cages may be related to the abundant food resources. Other factors, not evaluated during the fieldwork of 2013 and 2014, which may affect the parameter b include sex, stomach fullness (Froese 2006).

During the fieldwork of 2013 and 2014, there was evidence to indicate (albeit anecdotal) that some species might be affected by high mortality rates around the cages as a result of increased predation. Top predators such as seals and seabirds were also noted around the sea cages in particular when mackerel schools were swimming around the cages (anecdotal account). Fish may respond to high predation rates by decreasing their levels of activity as an adaptation that would lower the chances of encountering a predator (see Johansson and Andersson 2009). The decreased inactivity may result in the redirection of energy from maintenance into growth and reproduction (see Johansson and Andersson 2009). On the other hand, the presence of predators may induce physiological stress and thus the reduced activity of the prey may result in poor growth (Johansson and Andersson 2009).

The presence of an abundant food resource in the environment can reduce competition and therefore predation risk or mortality rates of some prey (Oro et al. 2013). However, in some cases animals may prefer a habitat of poorer quality to avoid the predation risks in a rich habitat. For example, Morris (2005) conducted a two-year experimental study and reported that despite the presence of supplemental food small mammals avoided these resources because of the presence of omnivorous predators. Thus, what may appear (e.g. habitat) to be of benefit for some species may be of cost to others which may be the case for wild fish around sea cages.

Results of the fieldwork in 2013 and 2014, indicated that sea cages provide a rich habitat for the sampled fish which leads to better overall condition with stonger impacts for whiting and saithe than mackerel. On the other hand, fish farming can potentially deter wild fish such as cod from reaching their spawning grounds which can result in decreased spawning success. For example, in experimental olfactory set up cod avoided the smell of fish farms. Thus, fish farms could be avoided by species such as cod which have high fidelity to their spawning grounds (Bjørn et al. 2005 cited in Dempster and Sanchez-Jerez 2008).

There is insufficient number of studies evaluating whether artificial structures in marine environments act as ecological traps or population sources for fishes (see also Chapter 1). Reubens et al. (2014) reported that offshore wind farms did not act as ecological traps for fish associated with the structures but rather increased their production (biomass) at the local level. Similarly, Dempster et al. (2011) evaluated whether coastal fish farms act as ecological traps by comparing the diets, conditions and parasite loads of cod and saithe sampled near and away from fish farms in nine locations along the coast of Norway. The authors concluded that fish farms are population sources for the species caught near the farms. On the othe hand, Hallier and Gaertner (2008) noted that fish aggregating devices can act as ecological traps for tuna.

Detecting the presence of an ecological trap is challenging as it is not always easy to clearly demonstrate whether an animal chooses a habitat or it is associated with the habitat (Hale and Swearer 2016). As Hale and Swearer (2016) pointed out this may lead to misidentification of some habitats as traps or not detecting ecological traps at all when in fact they might be present. In their review, Hale and Swearer (2016) suggested the use of experimental studies to detect preference but as this may not be always feasible a combination of approaches might be useful. The use of control/impact approach in detecting ecological traps is limited to the natural variability between sites. Therefore, either increase in the number of impacted sites might be appropriate and/or evaluating before and after impacts (Hale and Swearer 2016).

In this research, it was not possible to evaluate before/after impact of the fish farms on the sampled fish. Thus, drawing any broad inferences from this study is limited. The study was based only on two fish farms, limited number of samples, and only during the summer months and thus caution should be taken in extrapolating to all fish farms.

The linear mixed effects models for mackerel and whiting variables (e.g. length, mass and FCI) showed some deviation from homogeneity of variances and skewed

distributions. Despite some of these moderate failure in the model assumptions the models were kept as they provided sufficient information of the impact of the two fish farms on the sampled fish. As in Chapters 4 and 5, the models indicated that some fish are more residential around the sea cages and thus the two fish farms would have a stronger impact on these fish than on new arrivals. Moreover, some of the fish sampled near the sea cages were more strongly impacted than others because some fish were sampled near a salmon farm and others near a halibut farm which have a different impact on the wild fish.

6.5 Conclusions

Based on this chapter there is no empirical evidence to conclude that the selected fish farms act as ecological traps for all three sampled species. Abundance of food near the cages and improved condition in some fish indicated that young and adult fish may benefit in terms of faster growth and higher reproductive output.

As empirical studies are limited in exploring a combination of potential positive and negative impacts that may cause a habitat to act as an ecological trap or a population source at the population level, modelling approaches can be useful in such cases. This concludes Part I of the thesis on empirical studies related to exploring the influence of two fish farms on mackerel, whiting and saithe sampled near the sea cages. I present modelling work that builds on this empirical work in Part II of the thesis (Chapters 7 and 8).

PART II: MODELLING STUDIES

CHAPTER 7
A PHASE SPACE MODEL FOR EVALUATING THE POSITIVE AND NEGATIVE EFFECTS OF FISH FARMING ON WILD FISH

7.1 Introduction

Based on results from Chapters 4-6 fish farming provides a habitat rich in food resources that probably has a positive impact in terms of improved biological condition (possible proxy for reproductive output) on wild fish visiting the sea cages. On the other hand, observations during fieldwork indicated that there are potential negative effects on wild fish around sea cages in terms of increased predation rates. Although empirical evidence collected in Chapters 4-6 provides some information on the positive and negative effects of fish farming data cannot reveal how these effects interact and what the combination of these are on the population growth rates. When the quality of the new habitat improves reproduction and survival of individuals then the habitat may act as a population source. On the other hand if the habitat appears to be of high quality but it causes lower reproductive performance and survival (e.g. via increase in diseases, predation) then the habitat acts as an ecological trap (reviewed by Battin 2004) (see Chapter 6).

Fish farms are not alone in altering habitats by human activities. Marine renewable energy installations, artificial reefs, oil platforms amongst others can have a combination of positive and negative effects. Within Scottish waters, MASTS (Marine Alliance for Science and Technology for Scotland) identifies some of these research topics of particular importance[5]. For example, marine renewable energy installations, such as wind power devices, can have a range of potential positive and negative impacts on marine organisms (reviewed by Inger et al. 2009). Positive and negative factors can interact with one another in complex and often unpredictable ways which suggests the need for general methods for weighing up positive and negative environmental impacts, and especially to quantify uncertainties in these.

Evaluating the overall impact of positive and negative effects on marine organisms is difficult as the different ecological processes involved are hard to measure and it is

[5] http://www.masts.ac.uk/research/research-forums/ [Accessed: 13 May 2018].

particularly difficult to quantify distinct processes (e.g. mortality and fecundity) in terms that allow them to be compared. Precise predictions at the population level are impossible. It is helpful to explore 'what-if' scenarios to gain better understanding and identify knowledge gaps. Therefore, in this chapter a simple model is developed that allows the exploration of hypothetical combinations of positive and negative population effects of fish farming on wild fish in the vicinities of the farms. The model was applied to whiting and mackerel as both species were found in high numbers near the sea cages during the fieldwork undertaken in the summers of 2013-2014 (see Chapter 3).

7.2 Methods

The following section describes the methods used for building a model for each fish species. A phase space model is introduced, followed by introducing a well-known population model (Leslie population matrix model) used to build the underlying population model, and finally the case studies for whiting and mackerel are presented.

7.2.1 Basic phase space model

The model takes the form of a phase space with hypothesised positive effects on one axis and negative on the other (Figure 7.1). The population in its baseline state is in the bottom left, and the phase space is coloured according to overall population growth rate (the balance of positive and negative effect) allowing us to see which combinations of effects lead to overall positive or negative impacts on the population relative to the baseline state. The extent of the axes is chosen to encompass the maximum size of effect deemed possible but is essentially an arbitrary limit and the space could be extended to any extent (e.g. for an increase in fecundity we may go from no effect to an increase by 50% of the natural fecundity). The bottom right corner of the model represents the combination of maximum positive effects and no negatve effects, the top right corner represents the combination of maximum positive and negative effects and the top left corner represents maximum negative effects and zero positive effects. The bottom right corner of the model represents the population growth rate with no impacts.

The Leslie population matrix was used to build the phase space model as it is a well-established and validated model and it is relatively simple to build using very few parameters. The population dynamics are described at each point in the phase space model by a Leslie matrix model (explained in more detail in the next section), and the

overall dynamics are captured by the resulting intrinsic population growth rate at each point. For every point in the phase space the resulting population growth rate is calculated from the combination of positive and negative effects. Plotting the results for a range of expected positive and negative effects gives an indication of the likely overall effects, the likelihood of negative effects, and an elasticity analysis that contributes towards the understanding of which parameters are most important to understand.

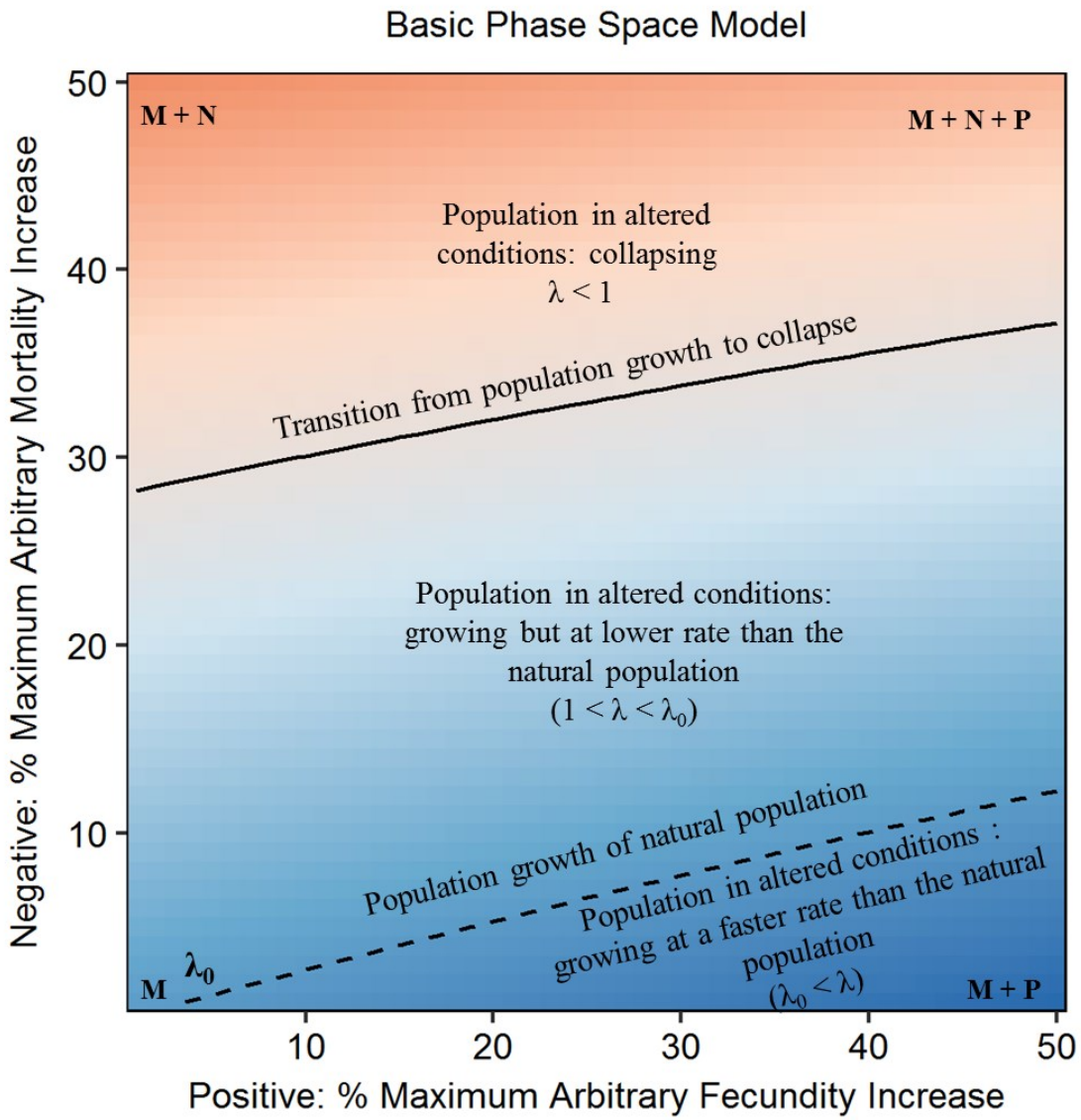

Figure 7.1 An example of a phase space model of a hypothetical fish population experiencing positive effects (improved fecundity) and negative effects (decreased survival). Bottom left corner represents the intrinsic growth rate of the natural population (λ_0) and is calculated by basic matrix M. Bottom right corner is a combination of M and a positive matrix P and the upper left corner is an M and a negative matrix N. Dashed contour is same intrinsic growth rate of natural population, points below dashed contour have a greater growth rate ($\lambda_0 < \lambda$). Solid contour represents transition from population growth to collapse. All points in upper grey region have negative growth ($\lambda < 1$). For most of the modelled phase space, population growth rate is lower than the natural rate ($1 < \lambda < \lambda_0$), and it would be necessary to show that the actual effects lie towards the bottom right (high positive, low negative effect) to be assured that overall effects are not negative.

7.2.2 Leslie population models

In order to weigh the positive (potential increase in fecundity) and negative (potential increase in mortality) effects on the wild fish in the vicinities of fish farms a widely used basic Leslie population model (Lewis 1942; Leslie 1945; Caswell 2001) was built for each species. Matrix population models are popular tools used in understanding animal population dynamics (Caswell 2001). Leslie population matrix models can be used for exploring population dynamics under various exploitation scenarios which can provide analysis of long term sustainability of the population. The basic Leslie population model uses estimates for age-specific survival rates and fertility rates to obtain the intrinsic growth rate of the population (Lewis 1942; Leslie 1945; Caswell 2001). Popularity of Leslie matrix population models amongst fisheries and conservation biologist lies in the easy model building (Caswell 2001). The basic model is written as:

$$N_{t+1} = M \times N_t \qquad (eq.7.1)$$

where N_t is a population vector which describes the number of individuals in each age class at time t, N_{t+1} is a population vector in the next year, and M represents the Leslie matrix. The mean survival and fertility at age were entered into a female only (assuming males do not affect spawning ability of females) Leslie population matrix (M) (Caswell 2001). The basic population can be found in equation 7.2

$$M = \begin{pmatrix} f_1 & f_2 & \cdots & f_n & f_{n+} \\ s_1 & 0 & \cdots & 0 & 0 \\ 0 & s_2 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & s_n & s_{n+} \end{pmatrix} \qquad (eq.7.2)$$

where s_i and f_i denote the age-specific survival rates and fertility rates of individuals, respectively.

7.2.2.1 Population growth rates and stable age structure

The population growth rate output of the models is the instantaneous rate which does not indicate how it will change as the overall density changes. Growth of a population is density-dependent when the growth rate changes as a function of the density of individuals in a population. Competition and predation can potentially regulate population growth (reviewed by Hixon et al. 2002).

Density-dependent competition is the result of the increased population size and fecundity and survival rates will increase or decrease based on actual or potentially limiting resources available to organisms which makes exponential growth less likely to take place. Population regulation by predation is the increase in prey mortality as a result of increased prey densities (Hixon et al. 2002). Predation is not always density dependent as there needs to be changes in the behavioural and developmental responses to changes in prey abundance (reviewed by Hixon et al. 2002). Density-dependent growth was omitted from this study as the focus is on the overall positive and negative effects of the current state of the population.

The model is deterministic and thus no stochasticity in survival and fertility rates was considered. The model also assumes closed population because there is lack of data on rates of immigration and emigration (Caswell 2001). In natural populations the assumption that survival, fertility and migration are stable fails and thus the development of more complex data-intensive stochastic alternative models are often explored. However, no level of model complexity can truly represent biological processes or system (see Ezard et al. 2010 and references therein).

The instinsic growth rate and stable age structure are used in studying change in population over time with the aim of predicting whether the population is increasing, decreasing or remaining constant. They satisfy the equation for the matrix M.

$$N_{t+1} = \lambda \times N_t \qquad (eq.7.3)$$

If $\lambda = 1$ the population remains constant, if $\lambda > 1$ the population grows (it is an instantaneous rate not continuous) and if $\lambda < 1$ the population declines over time (Caswell 2001). Thus, the overall rate of population growth is the dominant eigenvalue (λ). The corresponding eigenvector gives stable distribution of the population between classes which is represented by the right and left eigenvectors.

7.2.3 Parameterization of basic matrices

Based on the observations during fieldwork, results from the empirical studies and the literature (see Chapters 1, 4, 5, 6) indicated that there is potential, in theory, for fish farms to affect wild fish positively, negatively or none. I chose fecundity as a potential positive effect and mortality as potential negative effect to fit in the hypothetical model described in subsection 7.2.1. Improved condition was noted for mackerel and some of the gadoids and based on this (and literature) it is assumed that there might be potential increase in fecundity. It is worth noting that although condition may increase there may be decrease in the food quality. This has not been considered in the model as the aim of the model was to take into account two opposing effects and predict what may happen to the population when both effects take place. The choice of mortality as a negative effect in the model was based on observations during fieldwork. During fieldwork, schools of mackerel were noted to chase on schools of clupeids. This was also recorded in the underwater videos (see Appendix A). Bigger predators such as seals and seabirds were noted as well, particularly, when mackerel was around. Based on the presence of big predators around the sea cages it was assumed that predation would be also present for whiting. This has not been quantified and it is only anecdotal.

To build the phase space models for mackerel and whiting basic Leslie population models were build initially for each species. The final parametrised matrices for mackerel and whiting can be found in Tables 7.1 and 7.2, respectively.

7.2.3.1 Mackerel

Mackerel was found as one of the dominant species sampled near two fish farms and therefore chosen as a model species in this chapter (see Chapter 3).

The time of spawning in the Northeast Atlantic mackerel depends on the region; January in the Mediterranean Sea, February off the Portuguese coasts and ends in July north of Scotland and in the North Sea (Jansen and Gislason 2013). Maturity in mackerel is at around 2-3 years of age and a potential lifespan of over 20 years (Lockwood 1988; Jansen and Gislason 2013). Mackerel is a batch spawner (eggs released in batches) (Watson et al. 1992) and has a determinate fecundity (total fecundity is fixed before spawning) (Greer-Walker et al. 1994).

The chosen population matrix was a 12×12+ ages which was selected based on the data available for the different ages (Table 7.1). To parametrise the basic population

matrix, data on age specific abundances was obtained from ICES (2014a) assessment for the mackerel stocks. Data for the model was extracted for the three most recent years (2012-2014). The survival rate is the probability of the individual fish in each age class to survive to the next age class and can be calculated from:

$$s_i = \frac{N_i}{N_{(i-1)}} \qquad (eq.7.4)$$

where N_i is the number of individuals in the population at a given time. The survival rate was estimated for each age class for each year. The final survival rate was then averaged for each age class over the three years.

Age-specific fertility (or actual reproductive performance; Caswell 2001) is presented in the first row of the Leslie population matrix which refers to the number of offspring of a female of age *i* that will survive to the next age class *i* + *1*. Age specific fertilities for fish were calculated from the age-specific fecundities. Fecundity is the maximum reproductive output by females in a population (Caswell 2001). To calculate the fecundity at age for mackerel the following general equation was used (Wootton 1998):

$$Fecundity_i = a \times L_i^b \qquad (eq.7.5)$$

The parameters *a* (0.040) and *b* (4.480) were obtained from FishBase[6]. Length at age was obtained from west coast Scottish survey (SWC-IBTS) for the years 2012-2014 downloaded from the ICES database DATRAS[7] (see also Appendix A). The average number of males for the three years was 545 females and 474 males (total fish in the data = 991). Based on this it was assumed that the females account for about 50% of the population. Assuming that all eggs spawned are fertilized, the age-specific fertility is obtained by the following equation:

$$Fi = \frac{1}{2} \times Fecundity_i \times S_o \qquad (eq.7.6)$$

[6] www.fishbase.org [Accessed: 04 February 2018].
[7] http://www.ices.dk/marine-data/data-portals/Pages/DATRAS.aspx [Accessed: 04 February 2018].

where S_o is the survival from egg to age 1. A birth-pulse population (reproduction occurs over brief period of time) and a pre-breeding (young of the year not present) census were assumed (Caswell 2001). Using a pre-breeding approach allows the eggs and larvae to be included in the total reproductive value of the population and therefore the model starts at age 1. Marine larval survival is assumed to be low because high mortality rates occur before it reaches coastal waters and therefore it is typically of the order of 10^{-5} or less (see Artzrouni et al. 2014 and references therein).

Table 7.1 Parameterised population matrix (12×12+) age-based model for mackerel.

$$M_{mackerel} = \begin{bmatrix} 0.11 & 0.36 & 0.70 & 1.03 & 1.37 & 1.53 & 2.02 & 2.10 & 2.33 & 2.60 & 3.60 & 4.16 \\ 0.86 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.82 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.76 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.76 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.76 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.85 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0.84 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.72 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.71 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.69 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.81 & 0.71 \end{bmatrix}$$

7.2.3.2 Whiting

Whiting was the most common juvenile gadoid caught during the fieldwork of 2013 and 2014 and therefore was used as a model in this chapter (see Chapter 3). Whiting is a batch spawner with a relatively high fecundity (predicted fecundity for a fish of length of 45 cm = 1075-1298 thousands eggs) and protracted period ranging from 6 to 8 weeks period from January to September depending on locality (see Bailey et al. 2011). Most whiting mature by the age of 2 years (see Bailey et al. 2011).

The basic population matrix was a 7×7+ ages which was selected based on the data available (Table 7.1). To parameterise the whiting matrix survival probabilities were obtained using equation (7.4). It was assumed that roughly 50% of the population were females. Based on the data the number of females were 1890 out of total 3571 individuals. The number of males were 1681 out of 3571 individuals. The number at age were obtained from the ICES assessment (2014b) for the past three years (2012-2014) for the West Coast of Scotland. To obtain fecundity equation (7.5) was used and the parameters *a* (4.933) and *b* (3.25) were obtained for the Minch from Hislop and Hall (1974). Length

at age was obtained from west coast Scottish survey (SWC-IBTS) for the years 2012-2014 downloaded from the ICES database DATRAS. Fertility was calculated using equation (7.6).

Table 7.2 Parameterised population matrix (7×7+) age-based model for whiting.

$$M_{whiting} = \begin{bmatrix} 0.37 & 1.21 & 2.62 & 4.08 & 5.05 & 5.84 & 10.53 \\ 0.20 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.46 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.40 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.45 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.55 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.67 & 0.79 \end{bmatrix}$$

7.2.4 Scenarios for both mackerel and whiting found near sea cages

I explored different combinations of positive and negative effects and how this would affect the population growth rate λ of mackerel and whiting.

Two matrices were built, in addition to the basic matrices described in the previous section, one for hypothesised positive effects and one for the hypothesised negative effects. The positive matrix includes the hypothesised positive effect and in this case these are the potential increase in fecundity. The negative matrix includes the hypothesised negative effects and in this case these are the potential decrease in survival. A phase space model is built which is the combination of positive and negative effects or the combination of both the positive and negative matrices. On the x-axis, I plotted the magnitude of positive effect increase (e.g. fecundity) and on the y-axis I plotted the magnitude of the negative effect (e.g. mortality increase or survival decrease). Each point in the space is calculated by constructing a positive and a negative matrix and solving for the eigenvalue.

The model is run for a combination of zero to an upper limit multiplier, 0.5 in this case, of the positive and negative matrices. The choice of the upper limit is totally arbitrary and it is hypothesised that the farm impacts are unlikely to come anywhere close that value.

7.2.4.1 Mackerel

Based on results from fieldwork studies (see Chapters 4 and 5) mackerel visiting the cages ranged from 15.8 to 38.1 cm fork length (~ 0-11 years)(see Appendix A). Mackerel were caught during the summer months when they migrate to the feeding

grounds on the West Coast of Scotland. The fish farm impacts are assumed to be equally distributed amongst all age classes. Two matrices positive ($P_{mackerel}$) (Table 7.3) and a negative ($N_{mackerel}$) (Table 7.4) were built for positive and negative effects across all selected ages, respectively.

Table 7.3 Positive ($P_{mackerel}$) 12×12+ matrix for mackerel. This represents a 50% increase in the effect. The phase space model incorporates from zero to this arbitrary maximum.

$$P_{mackerel} = \begin{bmatrix} 0.06 & 0.18 & 0.35 & 0.52 & 0.69 & 0.77 & 1.01 & 1.05 & 1.17 & 1.30 & 1.80 & 2.08 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Table 7.4 Negative ($N_{mackerel}$) 12×12+ matrix for mackerel. This represents a 50% increase in the effect. The phase space model incorporates from zero to this arbitrary maximum.

$$N_{mackerel} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.43 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -0.41 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -0.38 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -0.38 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -0.38 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -0.43 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -0.42 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -0.36 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -0.36 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -0.35 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -0.41 & -0.41 \end{bmatrix}$$

7.2.4.2 Whiting

Based on results from fieldwork studies (Chapters 5) the whiting caught near cages ranged from 9.2 to 23.2 cm (~ 0-1 years) (see Appendix A). The age-class 2 was included in the model because the upper range of lengths sampled near the sea cages were also overlapping with the length range for the age-class 2. Although, whiting of age 2 were included in the model the main impact of sea cages on the whiting population is expected to be mainly on young fish. Two matrices, a positive ($P_{whiting}$) (Table 7.5) and a negative ($N_{whiting}$) (Table 7.6) were built for positive and negative effects, respectively.

Table 7.5 A positive ($P_{whiting}$) 7×7 matrix for whiting. This represents a 50% increase in the effect. The phase space model incorporates from zero to this arbitrary maximum.

$$P_{whiting} = \begin{bmatrix} 0.19 & 0.61 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Table 7.6 A negative ($N_{whiting}$) 7×7 matrix for whiting. This represents a 50% increase in the effect. The phase space model incorporates from zero to this arbitrary maximum.

$$N_{whiting} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.10 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -0.23 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

7.2.5 Matrix elasticities

As the estimates for the vital rates (e.g. fecundity, survival) for marine species are difficult to obtain it is important to incorporate the effect that uncertainty and variability in the vital rates can have on the population parameters (Caswell 2001). Elasticity (proportional change analysis) quantifies the proportional change in population growth rate for a proportional change in a given vital rate (fecundity, survival) (Benton and Grant 1999; Caswell 2001). Elasticities (e_{ij}) of λ with respect to a_{ij} or a matrix element can be calculated using:

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\eth \lambda}{\eth a_{ij}} = \frac{\eth log \lambda}{\eth log a_{ij}} \qquad (eq. 7.7)$$

Elasticities measure the linear change on a log scale or the slope of log λ plotted against log a_{ij}. Such information can be useful in understanding which ages are to be a focus of management or contribute most to fitness. For example, conservation efforts are needed if small changes in the vital rates affect the population growth rates. No

conservation efforts are needed when the changes in the vital rates do not affect or have limited effect on the population growth rates (Benton and Grant 1999).

7.2.6 Model implementation

To conduct all model population analyses, I used the open-source statistical software R (R Development Core Team 2016) run in RStudio (version 1.0.136, RStudio Team 2016). Some functions were used from the following packages popbio (Stubben and Milligan 2007), reshape2 (Wickham 2007), RColorBrewer (Neuwirth 2014), and ggplot2 (Wickham 2009). The R code for the phase space model is included in Appendix D.

7.3 Results

7.3.1 Population growth rate for mackerel and whiting under current state model

The population growth rate (λ) for mackerel was 1.35/year or 35% annual increase. The population growth rate (λ) for whiting was 1.09/year or 9% annual increase. The stable age distributions for mackerel and whiting are presented in Table 7.7. The highest proportion of the population for mackerel fall between the ages 1 and 3 whereas those for whiting are between 1 and 2 (Table 7.7).

Table 7.7 Stable age distributions for mackerel and whiting populations obtained from the basic matrices.

	Fraction of Population	
Age	**Mackerel**	**Whiting**
1	0.394	0.762
2	0.251	0.139
3	0.153	0.058
4	0.086	0.021
5	0.048	0.009
6	0.027	0.004
7+	0.017	0.007
8	0.011	-
9	0.006	-
10	0.003	-
11	0.002	-
12+	0.002	-

7.3.2 Phase space models for mackerel and whiting

The phase space models for mackerel and whiting are presented in Figures 7.2A, B. The phase space model for mackerel indicates that when the negative effects are at maximum and the positive effects are minimum (upper left corner of the phase space) the population growth rate is 0.81/year or 19% annual decrease. If the positive effects are at maximum and the negative effects are at minimum (lower right corner of the phase space model) the population growth rate is 1.48/year of 48% annual increase. If the positive and negative impacts are at a maximum (upper right corner of the model) the population growth rate is 0.92/year or decreasing by 8% annually.

The phase space model for whiting indicates that when the negative impacts are at maximum and positive at minimum (upper left corner of the phase space model) the population growth rate is 0.87/year or 13% annual decrease. If the positive and negative impacts are both maximum (upper right corner of the phase space model) then the annual population growth rate is 0.96/year or 4% annual increase. If the positive effects are maximum and negative effects are minimum (lower right corner of the phase space model) then the population growth rate is 1.21/year or there is 21% annual increase.

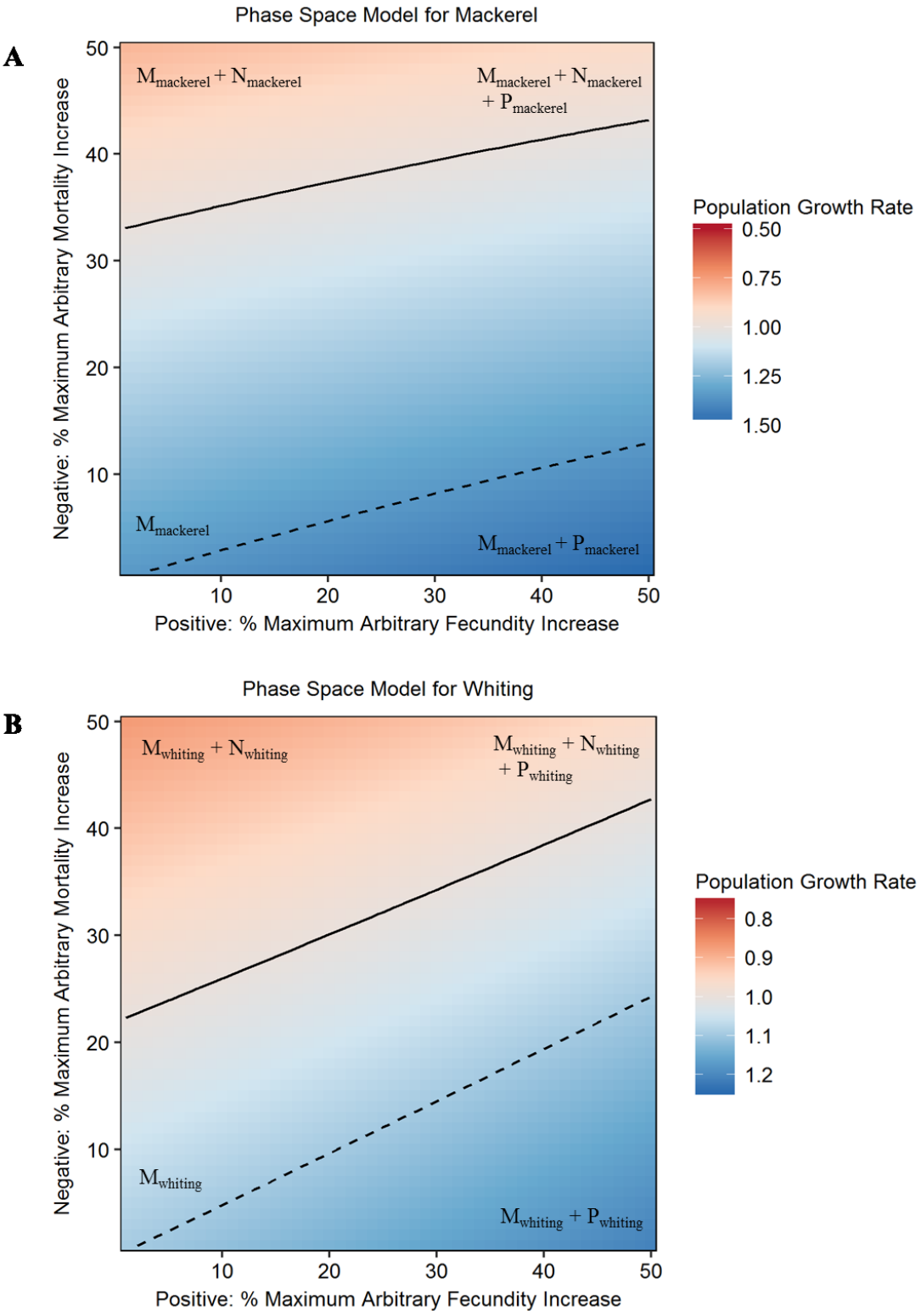

Figure 7.2 Phase space models for mackerel (A) and whiting (B) experiencing positive (improved fecundity) and negative (decreased survival) effects. Bottom left corner represents the intrinsic growth rate of the natural population from the basic matrix for each species. Dashed contour is same intrinsic growth rate of natural population, points below dashed contour have a greater growth rate.

7.3.3 Elasticity Analysis

7.3.3.1 Mackerel

The elasticity analysis shows that the survival probabilities contribute more to the population growth rate than that of the fertilities for all of the age classes (Table 7.8). The highest contributions are credited to survival probabilities of mackerel at age groups of 3 and younger. The total contribution to survival probabilities of ages 1, 2 and 3 years is 51.2%. The contribution to fertilities is highest at the ages of 2, 3, and 4 years. The elasticity peaks at 4.7% (3 years) and then declines. The overall contribution of survival and fertility to (λ) are 76.9% and 23.1%, respectively.

Table 7.8 Elasticity matrix ($E_{mackerel}$) of 12+ age classes for mackerel showing the proportional changes of fertility and survival rates that would contribute to changes in population growth rates.

$$E_{mackerel} = \begin{bmatrix} 0.02 & 0.04 & 0.05 & 0.04 & 0.03 & 0.02 & 0.02 & 0.01 & 0.01 & 0.00 & 0.00 & 0.00 \\ 0.21 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.17 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.13 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.09 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.06 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.04 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0.03 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.02 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.01 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.01 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.00 & 0.01 \end{bmatrix}$$

7.3.3.2 Whiting

The elasticity analysis shows that the survival probabilities contribute more to the population growth rate than that of the fertilities for all of the age classes (Table 7.9). The highest contributions are credited to survival probabilities of whiting at age groups of 2 and younger. The total contribution of survival of ages 1 and 2 years to the dominant eigenvalue (λ) is 38.5%. The contribution to fertilities is highest at the ages of 1 and 2 years. The overall contribution of survival and fertility to (λ) are 65.7% and 34.3%, respectively.

Table 7.9 Elasticity matrix ($E_{whiting}$) of 7+ age classes for whiting showing the proportional changes of fertility and survival rates that would contribute to changes in population growth rates.

$$E_{whiting} = \begin{bmatrix} 0.16 & 0.07 & 0.06 & 0.04 & 0.02 & 0.01 & 0.03 \\ 0.23 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.16 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.09 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.06 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.04 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.03 & 0.05 \end{bmatrix}$$

7.4 Discussion

The phase space models in this chapter were constructed to explore 'what-if' scenarios to determine possible impacts of fish farming on two fish species found near sea cages; migratory mackerel and resident whiting. Additionally, changes in fecundity and survival are measured in different units, but the changes in intrinsic growth rates allow a common way to compare them and to see which is likely to be a stronger effect. Although the phase space models are hypothesised representations of reality they provided an insight in what may happen in various scenarios that cannot be tested in the field. The model also provides information on what data are needed from the field in order to more accurately predict the outcomes.

7.4.1 Mackerel

7.4.1.1 Mackerel population dynamics

Population growth rate for mackerel in the current state was $\lambda = 1.35$/year or there is a 35% increase in population growth per year. This appears to be projected in accordance to trends in mackerel catches which have been on the rise since 2005. It is worth noting that this does not include other factors such as fishing effort or quotas. Based on mackerel egg surveys the total mass of fish in a stock that can reproduce (stock spawning biomass (SSB)) has been increasing. Between 2010 and 2013, a 30% increase in SSB of mackerel was noted (Barreto and Bailey 2015).

7.4.1.2 Interpretation of impacts on mackerel

The output of the phase space model for mackerel indicates that in the presence of both positive and negative effects the potential for population growth is higher than the population decline. This is assuming that both negative (increase in mortality because of predation or diseases) and positive effects (improved reproductive output) are equal in magnitude across all ages of mackerel visiting the fish farms. The model indicates that if the positive effects or the improved reproductive output of mackerel visiting the fish farms are stronger than the negative effects the population would benefit by growing at a rate of 48%/per year. Therefore, the fish farms would act as population sources. On the other hand when the negative effects (increase in mortality) near fish farms are stronger than the positive effects, the mackerel population growth rate would decrease by 19%/year. In this case the fish farms would act as ecological traps. As this is only a hypothetical scenario the reality can be anywhere between these two extremes.

If 82 sea lochs on the West Coast contain fish farms (Gillibrand et al. 2002) and the average mackerel biomass for the entire West Coast area is about 4.19 tonne/km^2 (total modelled area 110 000 km^2; Alexander et al. 2015) then the approximate proportion of mackerel biomass that would be affected by fish farming would be about 0.75%. Out of this 0.75%, some fish will be impacted more strongly than others. Based on the fatty acid analysis in Chapters 4 and 5, it was approximated that 26% of the mackerel that visited the cages were strongly impacted. This is based on the elevated levels of 18:2n-6 in the sample of mackerel that was analysed for FAs. Assuming the results apply to all the fish farms then 0.20% of the total mackerel population on the West Coast might be strongly affected by fish farming activities. Based on the phase space model the chances of having an overall positive impact are higher (~ 76.2%) than having an overall negative impact. The 76.2% estimation is based on the phase space model where 1905 observations (out of 2500 observations) on the phase space model had $\lambda > 1$. There is a 23.8% chance of the population to experience negative population growth (ecological trap) and 13.2% chance for the population visiting the sea cages to experience very high benefit (population source).

Based on the previous Chapter (Chapter 6), it was indicated that fish farming may act more as a population sources for mackerel. If this is the case then based on the phase space model about 26% of the mackerel population visiting the cages would increase in

growth between 35 and 48% per year. At a regional level this would apply to about 0.20% of the population.

It is also worth noting that in 2013, based on the FAs, about 36.5% of the analysed mackerel were strongly impacted whereas in 2014 about 18.2% for the fish visiting the halibut farm and 23.5% for those visiting the salmon farm were strongly impacted. In 2013, more fish waste was available at the halibut farm whereas there was less waste feed in 2014 (see Chapter 3). The direct fish farming impact on mackerel also depends on the fish farm.

7.4.2 Whiting

7.4.2.1 Whiting population dynamics

Population growth rate for whiting in the current state was $\lambda = 1.09$/year or there is a 9% increase in population growth per year. Although some increase in the stock spawning biomass of whiting (West Coast of Scotland) has been noted since 2005 the stock remains at low levels. Moreover, mortality of young whiting is high because the species is often caught as a bycatch with other species (e.g. Nephrops fisheries). Therefore, ICES advices to reduce the whiting catch to a minimum (Barreto and Bailey 2015).

7.4.2.2 Interpretation of impacts on whiting

Similar to mackerel, the output of the phase space model for whiting indicates that in the presence of both positive and negative effects the potential for population growth is higher than the population decline. This is assuming that the first two age classes of whiting are affected. The model indicates that if whiting population around fish farms are exposed to maximum levels of mortalities it will result in the decrease of the population growth rate by 13% per year. In this case the fish farms act as ecological traps mainly for young whiting. On the other hand if whiting benefit from the farms at an optimum level then the overall population would grow by 21% per year. Fish farms can act as population sources. These are extreme situations and as with mackerel the benefit and costs can be anywhere within the phase space model. There is a higher chance (64.8%) for whiting visiting the farm to be positively impacted than negatively. The 64.8% estimation is based on the phase space model where 1604 observations (out of 2500) on the phase space model had $\lambda > 1$. There is a 35.8% chance of the population to experience negative

population growth (ecological trap) and 26.1% chance for the population visiting the sea cages to experience very high benefit (population source).

If about 82 sea lochs on the West Coast contain fish farms (Gillibrand et al. 2002) and the average immature whiting biomass for the entire West Coast area is 0.287 tonne/km^2 (total modelled area 110 000 km^2; Alexander et al. 2015) then the approximate proportion of whiting biomass that may be affected by fish farming would be 0.73%. Out of this 0.73% some will be impacted more strongly than others.

Based on the FA analysis in Chapter 5, approximately 64.0% of the whiting visiting the sea cages were strongly impacted. This is based only on a sample of whiting that were used to be analysed for the FA. If the results are extrapolated to all sea lochs containing fish farms then about 0.47% of the total immature whiting population on the West coast of Scotland is likely to be stongly impacted. It is worth noting that in some lochs such as Loch Etive, whiting might be a resident population (see Bailey et al. 2011). If this is the case it is likely that all of the resident population in the loch has the potential to be impacted by the presence of fish farms.

Based on the empirical data for whiting there were no statistical differences in the condition of whiting sampled near and away from sea cages (Chapters 5-6). However, some individuals appeared to benefit in a positive way. Thus, if 64% of the whiting population that visits the sea cages benefits from the farms then the population would grow from 9 to 21% per year. At a regional level, 0.47% of the population would experience a population growth rate from 9 to 21% per year.

7.4.3 Species contrast

In both mackerel and whiting the hypothesised impact of fish farming appears to be more likely positive than negative. The overall positive effects of fish farming are stonger for mackerel than for whiting. Whiting has higher chances of experiencing negative impacts than mackerel. The potential differential impacts that mackerel and whiting experience when visiting the sea cages is potentially related to their behavioural differences. Mackerel is a migratory species and arrives on the West Coast of Scotland during the summer months to feed (Bailey et al. 2011). Mackerel enter various sea lochs where fish farms are present and is likely to benefit from these feeding excursions near the sea cages (see also Chapter 4-6). Mackerel of all sizes can be found visiting the fish farms; initially mackerel of greater length (older/bigger fish spawn earlier) arrive

followed by mackerel of smaller sizes (Lockwood 1988). It is likely that the older fish would benefit more from the fish farms than younger fish as they would spend more time feeding around the cages. However, in this model I assumed equal fish farming impacts across all ages because it is not clear how long all ages spend around the farms. Whiting on the other hand do not undertake long migrations. Juvenile whiting settle in inshore areas where fish farms are located and then move to deeper waters (Bailey et al. 2011). Thus, the juvenile stages are more likely to benefit from fish farms which would overall impact the population in a positive way.

Based on results of Chapters 4-6, the overall impacts of fish farming appear to have stonger effects on gadoid species (e.g. whiting) than on mackerel. This was supported by the models in this chapter where the overall negative effects are greater for whiting than for mackerel. It is also worth noting that the chances for both species to fall into an ecological trap are slightly higher than the chances of benefitting from the fish farms. This is based on the assumption that both positive and negative effects are equal which is less likely to be the case in reality.

Elasticity analysis for mackerel and whiting indicated that the population growth rates for both species are more strongly influenced by the survival rates of juvenile stages than the survival and fecundity of adult stages. Survival of the young stages is more important for the growth of the population than the fertility value of mature fish. As whiting sampled near the sea cages were mainly juveniles these fish would be more sensitive to positive/negative fish farming impacts. Bailey et al. (2011) noted that various human impacts (e.g. Nephrops fishing trawls, pollution) along the nearshore waters in Scotland can have a strong impact on the abundance of juvenile whiting populations. Based on the models in this study there is potential for fish farms to also affect the whiting population. However, further studies are needed to establish whether the impacts of fish farming are more positive or negative or none.

7.4.4 Limitation of the modelling approach

Models should be used with caution when used in providing advice on management and conservation of stocks. The strength of the model often lies in the quality of the data that is used to build it. Using limited data resources to build the models increase the uncertainty in the model outputs (Frisk et al. 2002). In the models used in this study there was uncertainty in the basic parameters (fecundity and survival). This is common for

marine species because of insufficient knowledge on mortality and reproductive ecology (Simon et al. 2012). There is very high uncertainty in estimating the true value of larval mortality which has high natural variation caused by factors such as starvation and predation (see Simon et al. 2012 and references therein). Thus, further development of the model should include natural variability of the parameters. Another limitation to the current model is the use of Leslie population matrix where the growth of the population is assumed to be density independent. In natural populations, however, the finite resources do not allow exponential growth. Further development of the model should include density dependence and other factors such as migration.

Scenarios in this chapter include an equal increase in fecundity (positive direction) and mortality (negative direction) which is unlikely to be the case in a real situation. The reality near the field is more complicated where the increase in fecundity and mortality is not equal.

In order to improve the model, estimates need to be obtained on the extent by which farming improves the reproductive output of wild fish around the sea cages. For example, laboratory studies can provide some insight into the extent by which fecundity of fish is improved when fed high energy diets. Fish near farms can also be sampled and ovaries examined and compared to those of fish from reference sites. The model can also be improved by providing a ratio of male and female fish visiting the sea cages. To estimate predation rates wild fish of interest around the fish farms can be tagged.

The single species modelling approach is simplistic as it does not include interaction with other species. For example, if some species benefit from the organic input from the farms another species may be replaced as a result of competition. Also, the increase in predation of one species by another means food for one and mortality for the other. Therefore, it is important to evaluate how fish farming directly affects wild fish around the fish farms but also to include indirect effects through the food web. In Chapter 8, I built an ecosystem-based model to evaluate the impacts of aquaculture activities on the mackerel and whiting populations in a sea loch.

7.5 Conclusions

The modelling approach used in this study was useful in exploring hypothetical scenarios of fish farming effects on wild fish when two potential antagonistic effects occur simultaneously. The models indicated that the overall positive effects are stronger

than the negative effects for both species. The whiting population visiting the sea cages is more likely to experience either very strong positive or negative effects than mackerel visiting the sea cages. Both mackerel and whiting have slightly higher chances in falling into an ecological trap than a population source. Based on empirical evidence and the literature fish farming can act as a population source for 26% and 64% of the mackerel and whiting populations visiting the sea cages, respectively. At a regional level, only 0.20% and 0.47% of the mackerel and whiting populations, respectively, would experience high growth rates.

CHAPTER 8
USING AN ECOSYSTEM-BASED APPROACH TO DETECT AQUACULTURE EFFECTS ON THE FOOD WEB IN A SEA LOCH

8.1 Introduction

Single species, age structured population models, used in the previous chapter (Chapter 7) were made using data for each species independently. Hence, the models do not capture effects caused by trophic interactions in the whole ecosystem. As well as affecting individuals of particular species through behavioural mechanisms, aquaculture also has a nutritional impact that flows throughout the ecosystem. It is important to consider trophic interactions and quantify trophic flows to address effects of aquaculture activities through species interactions, principally because changes in the abundance of one component of the ecosystem will change the constraints on other parts of the ecosystem. Ecosystem-based modelling approaches allow a more comprehensive understanding of effects of human exploitation on marine resource interactions (see Coll et al. 2013; Prato et al. 2014).

The aim of this study was to describe the ecological interactions in a sea loch (Loch Melfort) with and without aquaculture activities using Ecopath (see Chapter 2). Four scenarios were considered to explore the impacts of aquaculture activities in the Loch Melfort ecosystem: 1) ecosystem with aquaculture activities (is also the current state of the loch), 2) ecosystem with fish farming only, 3) ecosystem with mussel farming only, and 4) ecosystem with no aquaculture activities. Using these models, I also explored the impacts of fish farming on fish species such as whiting and mackerel sampled near one of the farms in the Loch (see Chapter 3). The models also indicate the direct and indirect impacts that the increase/decrease in biomass of different groups can have on other groups with and without aquaculture activities. Such knowledge is essential for future local management of the activities.

8.2 Methods

In this section, I describe the study site, and the general Ecopath model. I also describe how the model was built and the various input data. Once the basic Ecopath model was built and balanced the different scenarios were evaluated.

8.2.1 Study site

Loch Melfort was chosen as the study site for this Chapter because there was more observational data (see Chapter 3) available than for Loch Leven. Although, the fish farms in the loch have minimal production with respect to salmon farming the farms produce waste that have an impact on the ecosystem. The description of Loch Melfort can be found in Chapter 3. Observations of marine organisms during fieldwork can be found in Chapters 4 and 5 and Appendix A. The area that was selected for the study was approximately 10.1 km^2 (Figure 8.1).

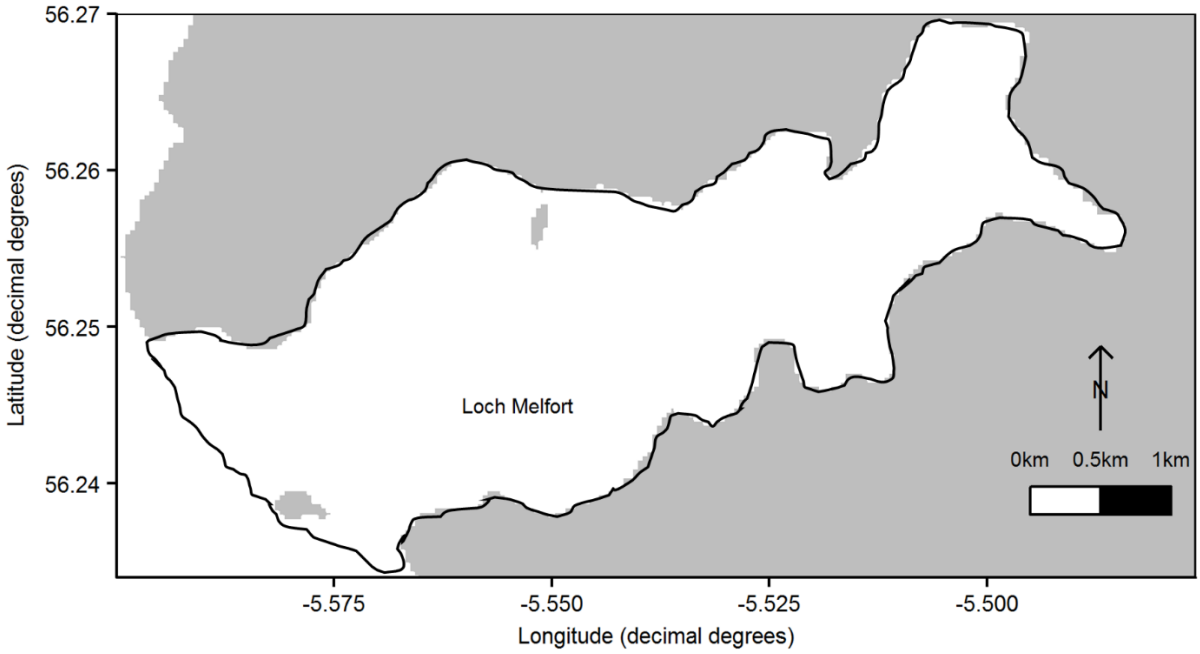

Figure 8.1 Map of Loch Melfort. The approximate area included in the model is outlined with black color.

8.2.2 Ecopath production and consumption

An Ecopath model is a snapshot of an ecosystem in terms of trophic interactions and energy flux in a particular period in time. Ecopath models divide the ecosystem into functional groups composed of either single species, life stages of a species (e.g. juvenile), or species with similar trophic levels and interactions. The functional groups are defined by the model designer based on the system of interest, and range from primary producers (e.g. phytoplankton, macrophytes) to top predators (e.g. seals). The main species and trophic levels that represent the studied ecosystem need to be included in an Ecopath model (Heymans et al. 2016). The inclusion of a minimum of one detritus, consumer and a top predator group is necessary in an Ecopath model (Heymans et al. 2016).

The Ecopath modelling approach is based on the principle that in a given time period the system is balanced so that production is equal to consumption (Polovina 1984). The total production is equal to the sum of total mass (or energy) removed by predation, non-predation losses (e.g. net biomass accumulation of the group, net migration of the group, mass flowing to detritus), and exports (e.g. fisheries).

Different functional groups are joined together through predator prey consumption. Consumption includes the production, non-assimilated food and respiration. The basic equation that represents the balance for each functional group *i* of the web is:

$$B_i \times \left(\frac{P}{B}\right)_i \times EE_i = \sum_j B_j \times \left(\frac{Q}{B}\right)_j \times DC_{ji} + EX_i \qquad (eq.8.1)$$

where B_i and B_j are the biomasses of prey (i) and predators (j), respectively; P is the production; P/B_i is the production to biomass ratio (in steady-state systems it is equal to instantaneous rate of total mortality (Z) (Allen 1971)); EE_i is the ecotrophic efficiency which is the proportion of total production of a group utilised in the system; Q is consumption; Q/Bj is the food consumption per unit biomass; DC_{ji} is the fraction of prey i in the average diet of predator j; EX_i is the export of compartment i towards other ecosystems such as net migration and harvest by fishery (Christensen and Walters 2004). For each functional group the Ecopath model requires estimates for B, P/B, Q/B ratios and diets. Fished groups require catches and discard inputs. EE is an output of the model and must be ≤ 1. However, when biomasses are not available EE values are used to allow the model to estimate the missing biomass parameters.

8.2.3 Loch Melfort models construction

Loch Melfort is considered an ecosystem which is defined as "any area of nature that includes living organisms and non-living substances interacting to produce an exchange of materials between the living and non-living parts…" (Odum 1959 cited in Tett 2008).

Static trophic network models were constructed for Loch Melfort using the Ecopath software (EwE; v.6.4.4.12634) (Polovina 1984; Christensen and Walters 2004). The following four scenarios were constructed: 1) impacts of both fish and mussel farming on the loch system and selected species (mackerel and whiting) (scenario 1; the current state of the loch); 2) impacts of only fish farming presence on the ecosystem and the selected species (mackerel and whiting) (scenario 2); 3) impacts of only mussel farming presence on the ecosystem and the selected species of interest (mackerel and whiting) (scenario 3); 4) the ecosystem with no aquaculture activities present (scenario 4). Each of these scenarios were constructed by adding components to scenario 4. For example, to consider only fish farming impacts on the ecosystem the mussel farming component was added from scenario 4. The models represent an annual average snapshot of the food web in the chosen system.

8.2.3.1 Loch Melfort functional groups

In a typical sea loch the nutrient dynamics are dependent on the balance between the flushing of nutrients in and out of the system (Ross et al. 1993). This dynamics is largely created by the combination of tidal circulation and the inflow of nutrient-rich water from rivers (Ross et al. 1993). Ross et al. (1993) suggested that the nutrient dynamics of a typical sea loch are similar to a laboratory chemostat. The researchers noted that in sea lochs, unlike in chemostats, there are temporary pulses of high levels of nutrients in the system which results in net export of nutrients (Ross et al. 1993). To simplify the model in this study, at the expense of reducing realism, the nutrient import and export is assumed to be in balance during the modelled period of one year.

As for the biotic components of the system the phytoplankton can be generated within the loch system and is also imported from the sea whereas the zooplankton and the carnivorous organisms immigrate from outside the system (jellyfish, fish larvae etc) (Ross et al. 1993). Irradiance and presence of zooplankton/carnivorous organisms regulate phytoplankton within the system (Ross et al. 1993). In this study, the

import/export of organisms is also assumed to be in balance and no seasonal patterns are taken into consideration. The model is assumed to be a snapshot of a static system.

In order to capture the food web in Loch Melfort all species must be included in the model. However, attempting to model every species individually is impossible and thus species and groups of species were defined based on taxonomic similarity and/or trophic group, with special cases chosen because of dominance in abundance, species of interest (e.g. mackerel and juvenile whiting), and fishing importance. Additionally, the functional groups included were based on a combination of previous Ecopath models for the West Coast of Scotland by Hagan and Pitcher (2005), Bailey et al. (2011), Alexander et al. (2015) and fieldwork observations carried out in Loch Melfort in 2013 and 2014. It is worth noting that although the maximum number of groups included are 14, other groups can be included (e.g. seals, skate, bacteria, other filter feeders etc.). Excluding some of these groups can affect the model results (see discussion), however, as this is the first attempt at modelling the food web of the loch the model is assumed to be a minimum realistic model.

For scenario 1, a total of 14 groups were included. These included: seabirds, mackerel, other fishes, juvenile whiting, crustaceans, echinoderms, polychaetes, zooplankton, farmed fish, farmed mussels, seaweed, phytoplankton, artificial feed, and detritus. For scenario 2, the same groups were present as in scenario 1, except mussel farming was removed. For scenario 3, the same groups were included as in scenario 1, except that fish farming and artificial feed were removed. For scenario 4, a total of 12 groups were used which were the same as in scenario 1, except fish farming, mussel farming and artificial feed were removed.

8.2.3.2 Model inputs

Three parameters, biomass, P/B, Q/B, and diet information are needed for each functional group. However, there was a lack of data for most functional groups and therefore some parameters were based on literature and previous models for the West Coast of Scotland (Hagan and Pitcher (2005) and Alexander et al. (2015)). To estimate most of the biomasses in the model EE was set at 0.95 which implies that the model explains 95% of the total mortality experienced by these groups by consumption via predators or fishery removal (Polovina 1984). Other sources of mortality (1-EE) not included in the model include diseases, senescence, etc. Information on functional groups

and parametrisation of the models can be found in Appendix F. All diet matrices can be found in Appendix G.

The Ecopath model needs to be balanced so that the energy input of all living groups is equal to the energy output. After all known parameters were entered in the software, the missing parameters were calculated by the software. At first, scenario 1 did not balance as the EE value for the farmed group was greater than 1 which indicated that the demand on them was too high. Thus, the P/B and Q/B values of the farmed group were decreased/increased to get the balanced model. For scenarios 2, 3 and 4 the balance of the models was achieved by adjusting the diets of some of the functional groups

8.2.4 Model analysis

A connectance diagram was generated by Ecopath to show the various relationships in the food web of Loch Melfort for each of the scenarios. The mean trophic level (TL) at which a group is receiving energy (Levine 1980) was calculated. The trophic level for primary producers is 1 and a fractional trophic level (TL of 1+, weighed average of the preys' TL) is given to consumer.

A number of ecological indicators can be used to give insight into how aquaculture activities impact the Loch Melfort ecosystem and how it compares to other ecosystems. Based on Odum's theory of development, an ecosystem that has not been disturbed by human activities evolve in sucession towards maturity where the system reaches stable state (Odum 1969). Odum (1969) presented 24 charactersitics that describe a mature system which can be estimated by Ecopath (Christensen 1995). In general, a mature system is described by an increase in biomass, detritus recycling, diversity in organisms and a complex food web. The indices selected in this study include: total system throughput (TST), total primary production/total respiration (PP/TR), total primary production/total biomass (PP/B), total biomass/total system throughput (B/TST), total biomass of the system (B), connectance and omnivory indices. I also considered cycling indices such as Finn's cycling index (FCI) and Finn's mean path length proposed by Finn (1976). The trophic fluxes are annual averages described in tonnes of wet weight/km^2.

The presence of anthropogenic activities such as aquaculture activities can have direct and indirect effects on the system. To detect the direct and indirect impacts of aquaculture activities on wild fish sampled near fish farms (juvenile whiting and mackerel) a mixed trophic impact analysis was implemented in Ecopath (MTI;

Ulanowicz and Puccia 1990). The mixed trophic impact (MTI) was calculated using the following formula:

$$MTIji = DCji - FCji \qquad (eq.8.2)$$

where i is the functional group in the diet of group j (DC_{ji}) and the proportion of predation on i due to predator j (FC_{ji}). MTI can be used as a sensitivity analysis (see Majkowski 1982) as it indicates the effect that a change in the biomass of one group will have on the biomass of other groups in a system (Ulanowicz and Puccia 1990). MTI was initially developed as an input and output method to evaluate economic interactions (Leontief 1951). All interactions are quantified by using matrices of relative net impacts (scaled between -1 and 1) which includes positive effects of prey on predator, negative effects of predator on prey and the indirect interactions of one group on another (see Coll et al. 2009). No predictions are made using MTI because abundance changes can lead to changes in diet compositions which are not included in the analysis[8].

8.3 Results

This section includes trophic structure and flows of the different scenarios (8.3.1), summary statistics of the models (8.3.2) and mixed trophic impact analysis (8.3.3). Diet matrices for all scenarios and additional model statistics output can be found in Appendix G.

8.3.1 Trophic structure and flow

Connectance diagrams for all scenarios are presented in Figures 8.2, 8.3, 8.4 and 8.5. All input parameters and those predicted by the model for all scenarios can be found in Tables 8.1, 8.2, 8.3 and 8.4.

8.3.1.1 Loch Melfort with fish and mussel farming (scenario 1)

The trophic levels for scenario 1, ranged from 1 for detritus to 3.92 for seabirds (Figure 8.2; Table 8.1). Artificial feed was at TL of 1 as it was considered non-living material similar to detritus. Trophic levels of farmed fish and mussel were 2 as both end

[8] http://sources.ecopath.org/trac/Ecopath/wiki/EwEugMixedTrophicImpact [Accessed: 04 February 2018].

up being harvested. The biomass of each group is represented by a circle and the size is proportional to the biomass in the ecosystem (Figure 8.2). The detritus group had the largest biomass (Figure 8.2). The total biomass of fish in the system was 2.49 tonnes/km^2 (Table 8.1). The total biomass of the macrobenthos (crustaceans, echinoderms, and polychaetes) was 59.22 tonnes/km^2 and that of the seaweed was 19.65 tonnes/km^2 (Table 8.1). The biomass of the farmed fish was 19.25 tonnes/km^2 and the artificial feed was at 35.59 tonnes/km^2 (Table 8.1). The biomass of the farmed mussels was 4.950 tonnes/km^2 (Table 8.1).

8.3.1.2 Loch Melfort with fish farming activity (scenario 2)

The connectance diagram for the food web of Loch Melfort with fish farming activity can be found in Figure 8.3. The trophic levels for scenario 2, ranged from 1 for detritus to 3.93 for seabirds (Figure 8.3; Table 8.2). The sum of the fish biomasses for the system was 2.804 tonnes/km^2/year (Table 8.2). The sum of the biomass of the macrobenthos (crustaceans, echinoderms, and polychaetes) was 68.54 tonnes/km^2(Table 8.2). The biomass of the polychaetes was the highest amongst the macrobenthos followed by the echinoderms (see Table 8.2).

8.3.1.3 Loch Melfort with mussel farming activity (scenario 3)

The food web for scenario 3 is presented in Figure 8.4. The trophic level of the seabirds was 4.05 (Figure 8.4; Table 8.3). The trophic level of the farmed mussel was 2.0 (Table 8.3). The sum of the biomass of the fish in the system was 2.292 tonnes/km^2 (Table 8.3). The total biomass of the macrobenthos (crustaceans, echinoderms, and polychaetes) was 55.709 tonnes/km^2 (Table 8.3). The biomass of the seaweed was predicted at 18.239 tonnes/km^2 (Table 8.3).

8.3.1.4 Loch Melfort with no aquaculture activities (scenario 4)

The food web for Loch Melfort without any aquaculture activity can be found in Figure 8.5. The trophic levels for scenario 4 ranged from 1 for detritus to 4.06 for seabirds (Table 8.4). Mackerel occupied a trophic level of 3.90 which is slightly higher than the range reported in Fishbase (TL: 3.63-3.73). Most of the biomass in the system is occupied by detritus and the macrobenthos (Table 8.4). The sum of the biomass of the macrobenthos (crustaceans, echinoderms, and polychaetes) was 64.959 tonnes/km^2

(Table 8.4). The biomass of the polychaetes was the highest amongst the macrobenthos followed by the echinoderms (see Table 8.4). The biomass of the seaweed was predicted at 20.825 tonnes/km^2 (Table 8.4).

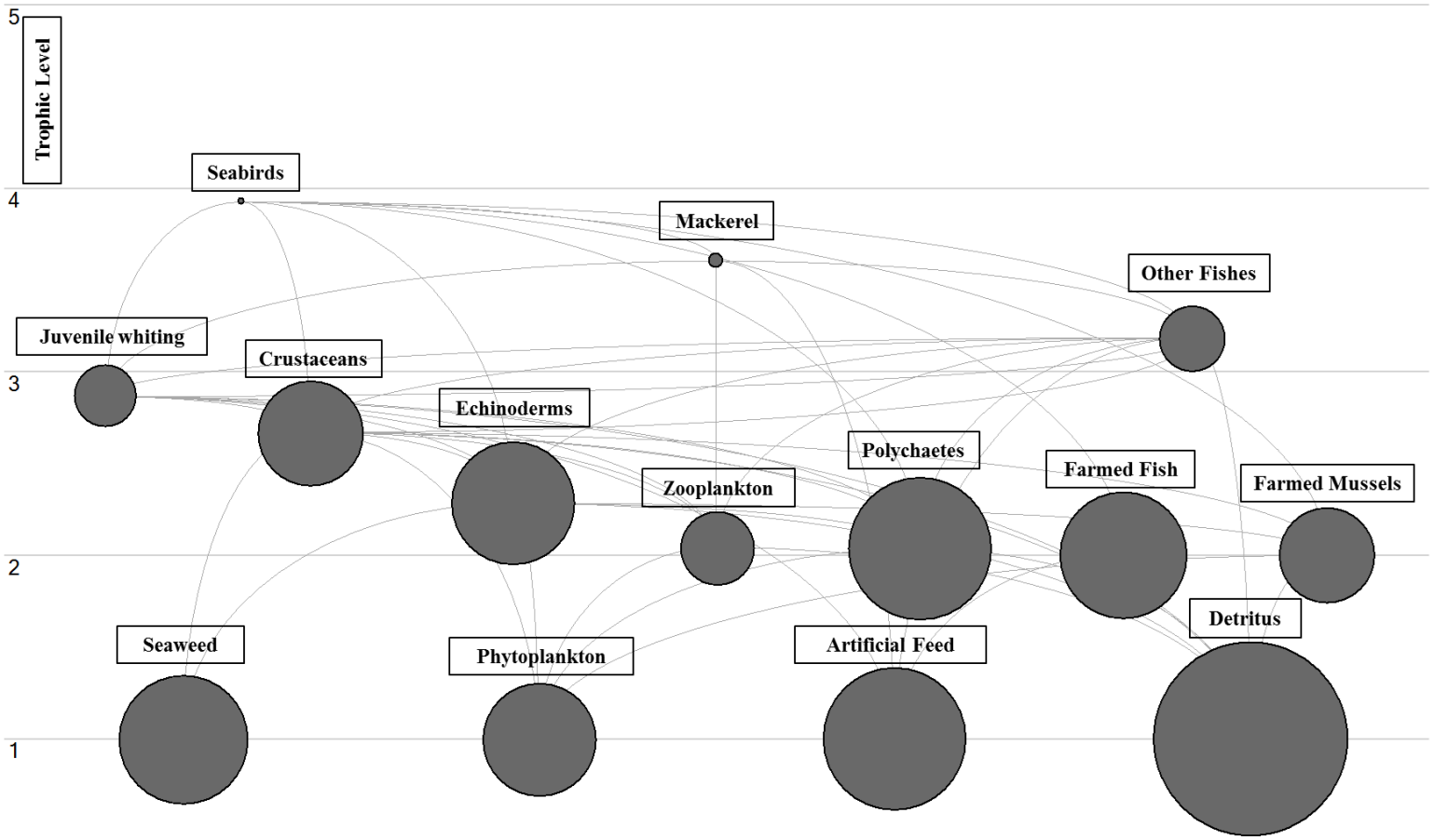

Figure 8.2 Connectance diagram for Loch Melfort with fish and mussel farming activities (scenario 1).

Table 8.1 Input parameters for the Loch Melfort ecosystem model in presence of both fish and mussel farming (scenario 1). Values in bold were predicted by the model.

	Group name	Trophic level	Biomass (tonnes/km^2) (B)	Production/ biomass (/year) (P/B)	Consumption/ biomass (/year) (Q/B)	Ecotrophic efficiency (EE)	Production/ consumption (P/Q)	Catches (tonnes/ km^2)
1	Seabirds	3.92	0.010	0.400	**26.667**	**0.000**	0.015	
2	Mackerel	3.60	**0.059**	0.690	4.400	0.950	**0.157**	0.0120
3	Other fishes	3.19	**1.298**	5.000	**16.667**	**0.729**	0.300	
4	Juvenile whiting	2.86	**1.128**	1.730	7.000	**0.129**	**0.247**	
5	Crustaceans	2.67	**7.198**	2.000	**13.333**	**0.837**	0.150	0.0220
6	Echinoderms	2.28	**16.269**	2.135	**14.233**	**0.943**	0.150	
7	Zooplankton	2.04	**1.866**	14.000	**46.667**	**0.948**	0.300	
8	Polychaetes	2.04	**35.755**	2.470	**16.467**	**0.949**	**0.150**	
9	Farmed Fish	2.00	19.25	1.450	1.830	**0.981**	**0.792**	27.380
10	Farmed Mussels	2.00	4.950	2.000	20.000	**0.581**	**0.100**	2.476
11	Seaweed	1.00	**19.652**	5.000		**0.500**		
12	Phytoplankton	1.00	10.0	70.000		0.800		
13	Artificial Feed	1.00	35.59			**0.875**		
14	Detritus	1.00	315.1			**0.815**		

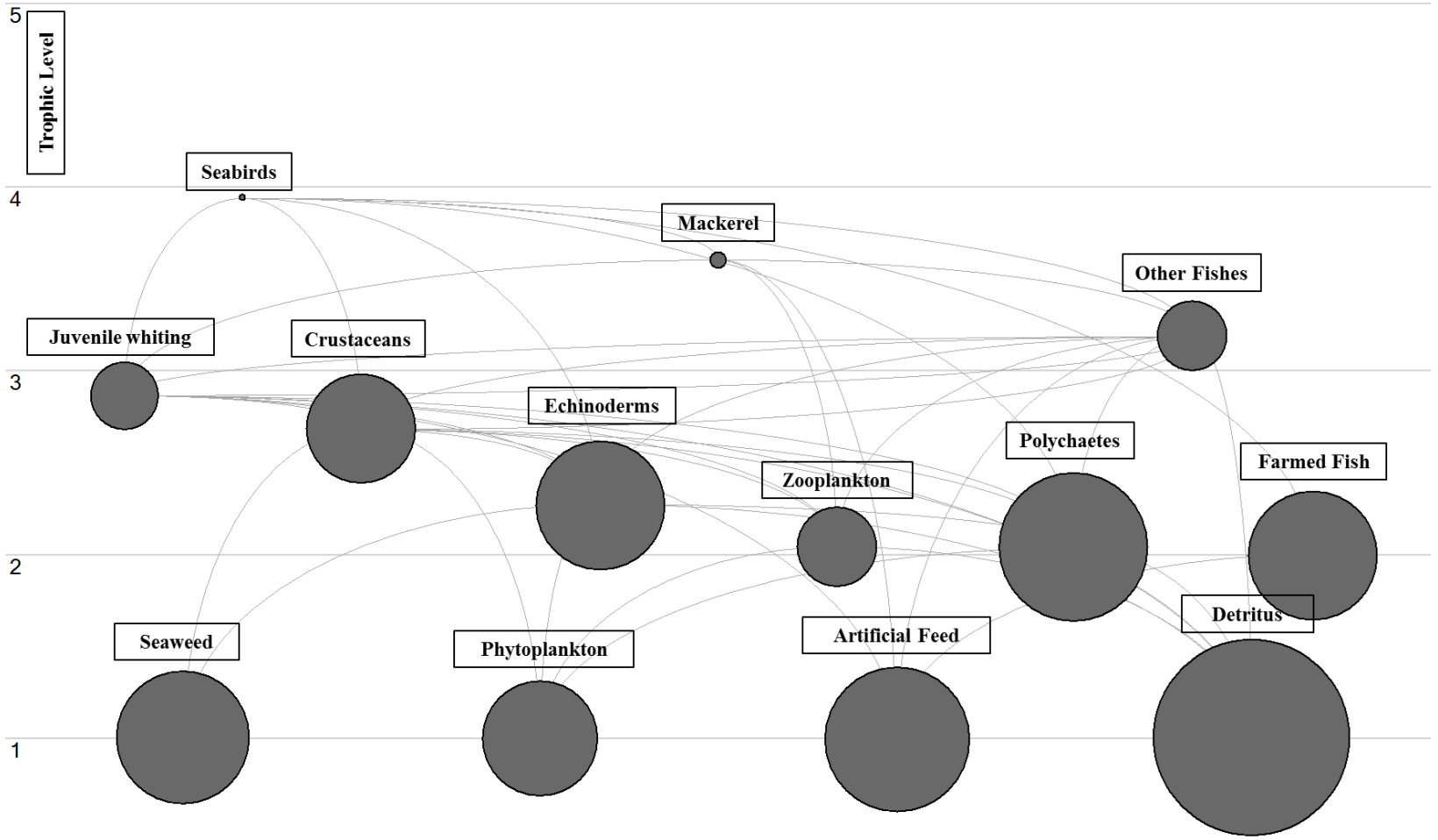

Figure 8.3 Connectance diagram for Loch Melfort with fish farming activity (scenario 2).

Table 8.2 Input parameters for the Loch Melfort ecosystem model in presence of fish farming only (scenario 2). Values in bold were predicted by the model.

	Group name	Trophic level	Biomass (tonnes/km^2) (B)	Production/ biomass (/year) (P/B)	Consumption/ biomass (/year) (Q/B)	Ecotrophic efficiency (EE)	Production/ consumption (P/Q)	Catches (tonnes/ km^2)
1	Seabirds	3.93	0.010	0.400	**26.667**	**0.000**	0.0150	
2	Mackerel	3.60	**0.059**	0.690	4.400	0.950	**0.157**	0.0120
3	Other fishes	3.19	**1.464**	5.000	**16.667**	**0.727**	0.300	
4	Juvenile whiting	2.87	**1.281**	1.730	7.000	0.950	**0.247**	
5	Crustaceans	2.68	**8.158**	2.000	**13.333**	**0.836**	0.150	0.0220
6	Echinoderms	2.27	**18.883**	2.135	**14.233**	**0.943**	0.150	
7	Zooplankton	2.04	**2.205**	14.00	**46.667**	**0.948**	0.300	
8	Polychaetes	2.04	**41.501**	2.470	**16.467**	**0.949**	**0.150**	
9	Farmed Fish	2.00	19.25	1.450	1.830	**0.981**	**0.792**	27.38
10	Seaweed	1.00	**22.652**	5.00		0.500		
11	Phytoplankton	1.00	10.0	70.00		0.800		
12	Artificial Feed	1.00	35.59			**0.886**		
13	Detritus	1.00	307.1			**0.859**		

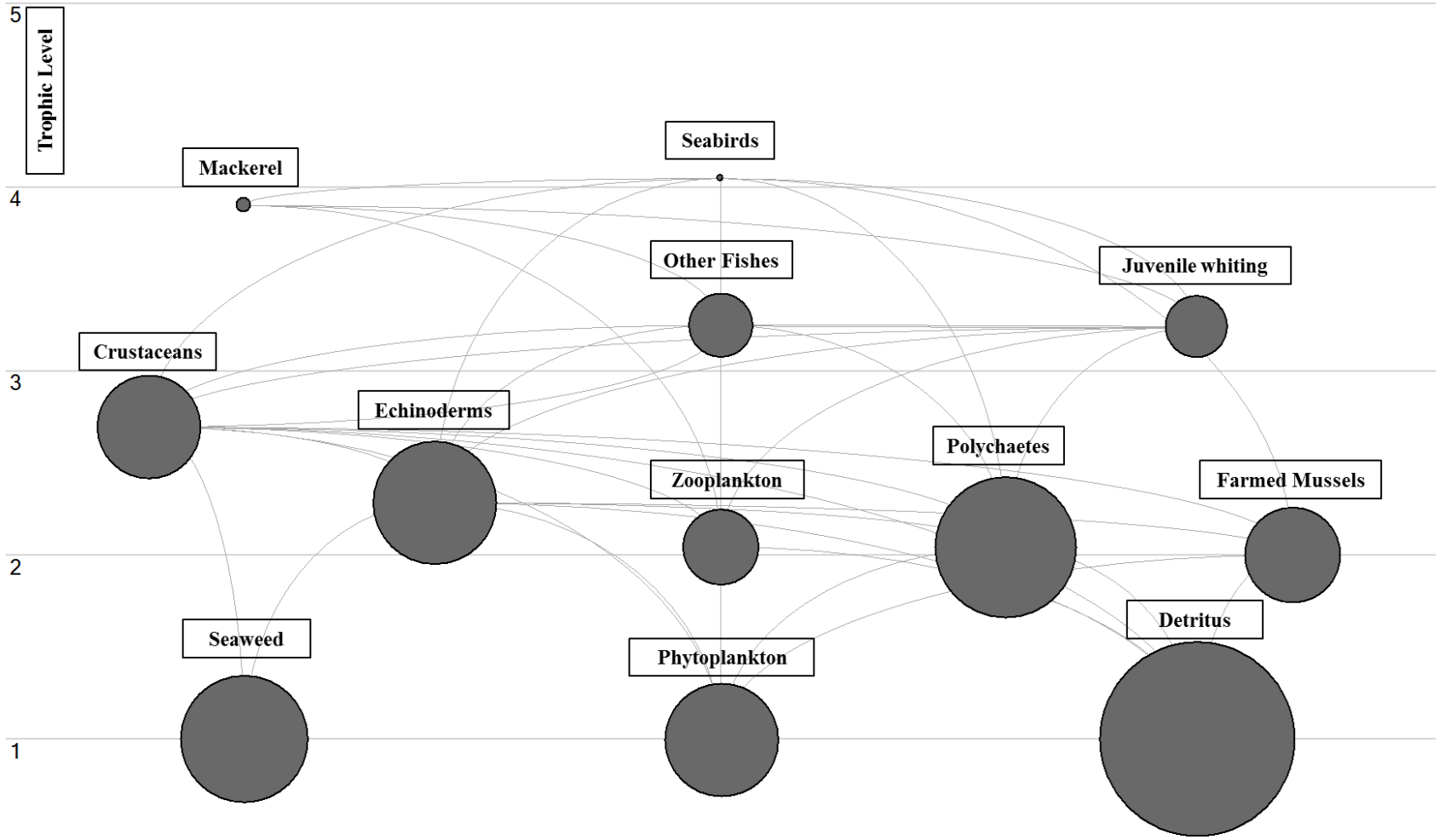

Figure 8.4 Connectance diagram for Loch Melfort with mussel farming activity (scenario 3).

Table 8.3 Input parameters for the Loch Melfort ecosystem model in presence of mussel farming only (scenario 3). Values in bold were predicted by the model.

	Group name	Trophic level	Biomass (tonnes/km^2) (B)	Production /biomass (/year) (P/B)	Consumption /biomass (/year) (Q/B)	Ecotrophic efficiency (EE)	Production/ consumption (P/Q)	Catches (tonnes/km^2)
1	Seabirds	4.05	0.010	0.400	**26.667**	**0.000**	0.0150	
2	Mackerel	3.90	**0.059**	0.690	4.400	0.950	**0.157**	0.012
3	Other fishes	3.25	**1.188**	5.000	**16.667**	**0.736**	0.300	
4	Juvenile whiting	3.24	**1.045**	1.730	7.000	**0.129**	**0.247**	
5	Crustaceans	2.70	**6.558**	2.000	**13.333**	**0.840**	0.150	0.022
6	Echinoderms	2.28	**15.213**	2.135	**14.233**	**0.943**	0.150	
7	Zooplankton	2.04	**2.019**	14.00	**46.667**	**0.948**	0.300	
8	Polychaetes	2.04	**33.938**	2.470	**16.467**	**0.949**	**0.150**	
9	Farmed Mussels	2.00	4.950	2.000	20.000	**0.557**	**0.100**	2.476
10	Seaweed	1.00	**18.239**	5.00		0.500		
11	Phytoplankton	1.00	10.000	70.00		0.800		
12	Detritus	1.00	308.0			**0.773**		

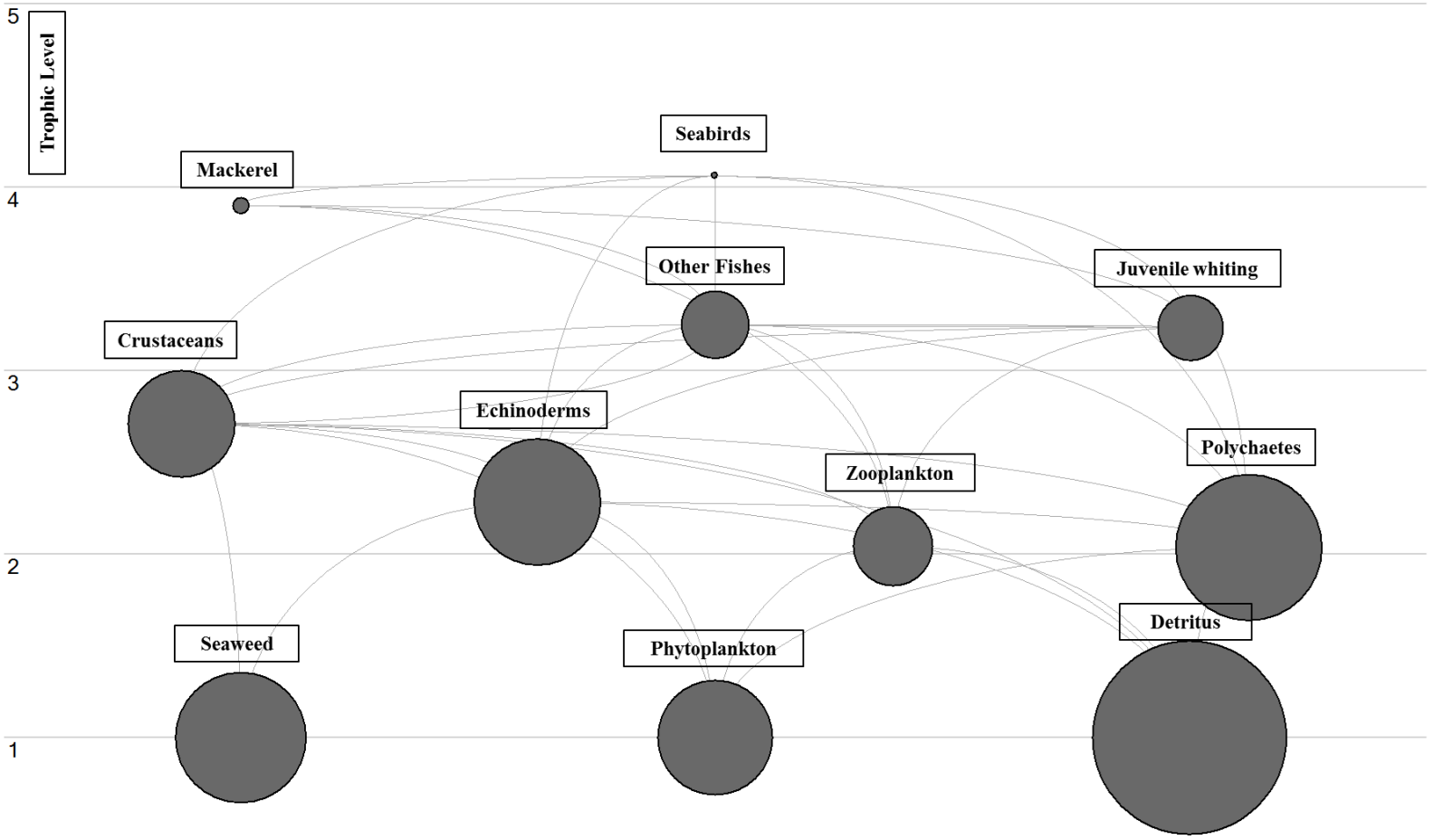

Figure 8.5 Connectance diagram for Loch Melfort with no aquaculture activity (scenario 4).

Table 8.4 Input parameters for the Loch Melfort ecosystem wth no aquaculture activities (scenario 4). Values in bold were predicted by the model.

	Group name	Trophic level	Biomass (tonnes/km^2) (B)	Production/ biomass (/year) (P/B)	Consumption/ biomass (/year) (Q/B)	Ecotrophic efficiency (EE)	Production/ consumption (P/Q)	Catches (tonnes/km^2)
1	Seabirds	4.06	0.010	0.400	**26.667**	**0.000**	0.015	
2	Mackerel	3.90	**0.059**	0.690	4.400	0.950	**0.157**	0.0120
3	Other fishes	3.25	**1.305**	5.000	**16.667**	**0.734**	0.300	
4	Juvenile whiting	3.24	**1.153**	1.730	7.000	**0.126**	**0.247**	
5	Crustaceans	2.71	**7.242**	3.750	**13.333**	**0.838**	0.150	0.022
6	Echinoderms	2.28	**17.601**	2.135	**14.233**	**0.944**	0.150	
7	Zooplankton	2.04	**2.308**	14.000	**46.667**	**0.949**	0.300	
8	Polychaetes	2.04	**40.116**	5.000	**16.467**	**0.949**	0.150	
9	Seaweed	1.00	**20.825**	5.000		**0.500**		
10	Phytoplankton	1.00	**10.000**	70.00		**0.800**		
11	Detritus	1.00	300			**0.820**		

8.3.2 Summary statistics and network flow indices

Selected summary statistics for all model scenarios can be found in Table 8.5. Full summary statistics for all model scenarios can be found in Appendix G.

8.3.2.1 Loch Melfort with fish and mussel farming (scenario 1)

The total system throughput which represents the size of the system in terms of flow which is the sum of total consumption, total export, total respiration, and total flow to detritus (Ulanowicz 1986). The total system throughput for scenario 1, was predicted at 2485.49 tonnes/km^2/year (Table 8.5). Another indicator for the state of the ecosystem is the primary production/respiration ratio (PP/R) (Odum 1969, 1971). If an ecosystem respires all the energy fixed by primary production then PP/R ~ 1. When organic energy is imported from outside the system then the PP/R ratio is < 1 and if the PP/R > 1 then there is export of energy fixed by primary producers (Odum 1969, 1971). The primary production to respiration ratio was 1.102 (Table 8.5). Primary production to total biomass ratio was 6.797 and the total biomass excluding detritus was 117.44 tonnes/km^2 (Table 8.5).

The connectance index is the number of actual links in relation to the number of theoretical links in the food web (Gardner and Ashby 1970). The connectance index for scenario 1 was 0.357 (Table 8.5). An alternative index to the connectance is the omnivory index which shows the extent to which the ecosystem shows weblike attributes (Christensen and Pauly 1993). Systems that are not disturbed by human activities tend to have more branched food web. The omnivory index for scenario 1 was 0.164 (Table 8.5).

Finn's cycling index (FCI) is the total proportion of the recycled flow in the ecosystem (Finn 1976). When a system is disrupted by human activities the cycles are short and fast whereas in a more complex system the cycles are long and slow (Odum 1969; Christensen and Pauly 1993; Christensen 1995). To quantify the length of each cycle Finn's mean path length represents mean number of groups that energy inflow passes through (Finn 1980). Diversity of flows and cycling affect the path lengths. Finn's cycling index for the food web in presence of both aquaculture activities (scenario 1) in Loch Melfort was 9.38 % of the total throughput (Table 8.5). The Finn's mean path length for scenario 1 was 2.598 (Table 8.5).

8.3.2.2 Loch Melfort with fish farming (scenario 2)

The total system throughput was estimated at 2583.93 tonnes/km^2/year (Table 8.5). The primary production to respiration ratio was 1.072 (Table 8.5). Mean trophic level of the catch was 2.001 (Table 8.5). Primary production to total biomass ratio was 6.483 and the total biomass excluding detritus was 125.46 tonnes/km^2 (Table 8.5). The connectance index was 0.380 and the system omnivory index was 0.189 (Table 8.5). Finn's cycling index was 10.59% of total throughput and Finn's mean path length was 3.021 (Table 8.5).

8.3.2.3 Loch Melfort with mussel farming (scenario 3)

The total system throughput was estimated at 2291.46 tonnes/km^2/year. The primary production to respiration ratio was 1.144 (Table 8.5). Mean trophic level of the catch was 2.015 (Table 8.5). Primary production to total biomass ratio was 8.487 and the total biomass excluding detritus was 93.22 tonnes/km^2 (Table 8.5). The connectance index was 0.364 and the system omnivory index was 0.142 (Table 8.5). Finn's cycling index was 9.23 of total throughput and Finn's mean path length was 2.903 (Table 8.5)

8.3.2.4 Loch Melfort no aquaculture activities (scenario 4)

The total system throughput was estimated at 2383.28 tonnes/km^2/year. The primary production to respiration ratio was 1.110 (Table 8.5). Mean trophic level of the catch was 3.131 (Table 8.5). Primary production to total biomass ratio was 7.992 and the total biomass excluding detritus was 100.62 tonnes/km^2 (Table 8.5). The connectance index was 0.390 and the system omnivory index was 0.168 (Table 8.5). Finn's cycling index was 10.55% of total throughput and Finn's mean path length was 2.968 (Table 8.5)

Table 8.5 Comparison of the Loch Melfort ecosystem scenarios and other ecosystems with fish and mussel farming.

	Model Scenarios				Other Ecosystems			
Parameters	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**	**Sardinia Island 1994 (before fish farming) (Díaz López et al. 2008)**	**Sardinia Island 2006 (after fish farming) (Díaz López et al. 2008)**	**Southeastern Spain (fish farming) (Bayle-Sempere et al. 2013)**	**Mont Saint Michel bay (mussel farming) (Leloup et al. 2008)**
Total system throughput (TST) (tonnes/km^2/year)	2485.49	2583.93	2291.46	2383.28	1730	3667	119601	9400
Total primary production/total respiration (TP/TR)	1.102	1.072	1.144	1.110	1.37	1.09	0.116	6.1
Net system production (tonnes/km^2/year)	74.01	54.42	99.61	79.57	110.34	62.63	-12207.29	3700
Total primary production/total biomass (TPP/TB)	6.797	6.482	8.487	7.992	7.79	4.61	0.204	24.6
Total biomass/total throughput (tonnes/km^2)	0.047	0.049	0.041	0.042	0.03	0.04	0.07	0.02
Total biomass (excluding detritus) (tonnes/km^2/year)	117.44	125.46	93.22	100.62	51.95	160.54	7864.55	180
Mean trophic level of the catch	2.001	2.001	2.015	3.131		2	2	2.11
Connectance index	0.357	0.380	0.364	0.390			0.19	0.17
System omnivory index	0.164	0.189	0.142	0.168	0.19	0.16	0.13	0.06
Finn's cycling index (FCI) (% of total throughput)	9.38	10.59	9.23	10.55	24.96	21.43		0.64
Finn's mean path length	2.598	3.021	2.903	2.968	4.27	3.88		2.10

8.3.3 Mixed trophic impact analysis

In scenario 1, seabirds have direct negative impact on mackerel and whiting (Table 8.6). Crustaceans have a negative impact on seabirds, mackerel and other fishes and positive effect on whiting (Table 8.6). Zooplankton has positive impact on seabirds, other fish and mackerel and negative impact on juvenile whiting (Table 8.6). Polychaetes have slight positive impact on seabirds and juvenile whiting but negative impact on mackerel and zooplankton (Table 8.6). The presence of farmed fish had slight negative effect on mackerel and whiting (Table 8.6). Farmed mussels had slight negative effect on seabirds, mackerel, zooplankton and polychaetes and a slight positive effect on juvenile whiting (Table 8.6). Phytoplankton had positive impact on almost all groups (Table 8.6). Seaweeds had slight negative impact on the mackerel and other fishes and slight positive impact on seabirds and juvenile whiting (Table 8.6). Artificial feed had positive impact on seabirds, mackerel and whiting (Table 8.6). Detritus had slight negative effect on mackerel and other fishes and positive effect on juvenile whiting, crustaceans and polychaetes (Table 8.6).

In scenario 2, seabirds have direct negative effect on mackerel and juvenile whiting (Table 8.7). Mackerel has a positive effect on seabirds and no apparent effect on juvenile whiting (Table 8.7). Crustaceans have negative impact on seabirds, mackerel and other fish and positive impact on juvenile whiting (Table 8.7). Echinoderms have positive impact on seabirds, mackerel and other fishes and a negative impact on polychaetes (Table 8.7). Zooplankton had positive impact on seabirds, mackerel, other fishes and negative impact on juvenile whiting (Table 8.7). Phytoplankton has positive impact on seabirds, mackerel and negative impact on juvenile whiting (Table 8.7). Detritus has slight positive impact on seabirds and whiting and a negative impact on mackerel and other fishes (Table 8.7).

In scenario 3, zooplankton had slightly positive impact on seabirds, mackerel, other fishes and juvenile whiting (Table 8.8). Phytoplankton had positive impact on seabirds, mackerel, other fishes and whiting (Table 8.8). The polychaetes had negative effect on almost all groups including a slight negative effect on juvenile whiting (Table 8.8). Mussel farming had slight negative impact on most groups (Table 8.8). In scenario 4, the group ‘other fish’ had strong positive effect on seabirds and mackerel and negative impact on juvenile whiting (Table 8.9). Zooplankton had strong positive effect on seabirds and

all fish (Table 8.9). Polychaetes had negative impact on almost all groups (Table 8.9). Phytoplankton had positive impact on almost all groups (Table 8.9).

Table 8.6 Mixed trophic impacts for functional groups of Loch Melfort ecosystem in scenario 1. An impact of a group is represented by a number in the table. Numbers in rows represent impacts of the impacting group and those in the columns are the impacted groups. Positive/negative values represent an increase/decrease in the biomass of the impacting group and the corresponding increase/decrease of the biomass of the impacted group.

Impacting/Impacted	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Seabirds	-0.06	-0.49	0.00	-0.11	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	-0.49	0.00	0.00
2. Mackerel	0.06	-0.28	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00	0.00
3. Other Fishes	0.32	0.19	-0.16	-0.76	-0.09	0.00	-0.21	0.01	0.01	0.02	0.03	0.02	0.03	0.01	-0.09	0.19	0.01	0.02
4. Juvenile whiting	0.09	-0.07	-0.04	0.00	-0.05	0.00	-0.01	0.00	-0.02	0.01	0.02	0.00	-0.06	0.00	-0.05	-0.07	-0.02	0.01
5. Crustaceans	-0.09	-0.14	-0.34	0.34	-0.36	-0.19	-0.12	0.01	-0.01	0.00	-0.05	0.03	-0.01	-0.01	0.64	-0.14	-0.01	0.00
6. Echinoderms	0.06	0.01	0.07	0.00	-0.01	-0.34	0.05	-0.24	0.00	-0.13	-0.47	0.09	0.00	0.01	-0.01	0.01	0.00	-0.13
7. Zooplankton	0.13	0.19	0.27	-0.13	0.01	-0.04	-0.24	-0.03	0.00	-0.04	0.02	-0.07	0.00	0.00	0.01	0.19	0.00	-0.04
8. Polychaetes	0.04	-0.07	-0.02	0.03	-0.03	-0.05	-0.24	-0.40	0.00	-0.19	0.04	-0.29	0.00	-0.36	-0.03	-0.07	0.00	-0.19
9. Farmed Fish	-0.01	-0.03	0.00	-0.09	0.01	0.00	0.00	0.00	-0.63	0.00	0.00	0.00	-0.33	0.00	0.01	-0.03	0.37	0.00
10. Farmed Mussels	-0.01	-0.01	-0.02	0.01	0.00	-0.01	-0.05	-0.03	0.00	-0.35	0.01	-0.08	0.00	0.01	0.00	-0.01	0.00	0.65
11. Seaweed	0.00	-0.02	-0.04	0.05	0.10	0.07	-0.01	-0.03	0.00	-0.02	-0.08	0.02	0.00	0.00	0.10	-0.02	0.00	-0.02
12. Phytoplankton	0.13	0.10	0.20	-0.07	0.02	0.09	0.45	0.23	0.00	0.42	-0.07	-0.27	0.00	-0.20	0.02	0.10	0.00	0.42
13. Artificial Feed	0.03	0.07	0.00	0.21	-0.01	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	-0.01	0.07	0.29	0.00
14. Detritus	0.04	-0.04	-0.03	0.12	0.16	0.15	-0.02	0.15	0.00	-0.06	-0.15	-0.09	-0.01	0.00	0.16	-0.04	0.00	-0.06
15. Crustacean Fisheries	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16. Recreational Fisheries	-0.02	-0.22	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.22	0.00	0.00
17. Farmed Fish Harvesting	0.01	0.03	0.00	0.09	-0.01	0.00	0.00	0.00	-0.37	0.00	0.00	0.00	0.33	0.00	-0.01	0.03	-0.37	0.00
18. Farmed Mussels Harvesting	0.00	0.01	0.01	0.00	0.00	0.00	0.02	0.01	0.00	-0.28	0.00	0.03	0.00	0.00	0.00	0.01	0.00	-0.28

Table 8.7 Mixed trophic impacts for functional groups of Loch Melfort ecosystem in scenario 2. An impact of a group is represented by a number in the table. Numbers in rows represent impacts of the impacting group and those in the columns are the impacted groups. Positive/negative values represent an increase/decrease in the biomass of the impacting group and the corresponding increase/decrease of the biomass of the impacted group.

Impacting/Impacted	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Seabirds	-0.06	-0.49	0.00	-0.10	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	-0.49	0.00
2. Mackerel	0.06	-0.28	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00
3. Other Fishes	0.34	0.19	-0.15	-0.77	-0.09	0.00	-0.20	0.01	0.01	0.03	0.02	0.04	0.01	-0.09	0.19	0.01
4. Juvenile whiting	0.09	-0.07	-0.04	0.00	-0.06	0.00	0.00	0.00	-0.02	0.02	0.00	-0.06	0.00	-0.06	-0.07	-0.02
5. Crustaceans	-0.09	-0.14	-0.35	0.35	-0.36	-0.19	-0.13	0.01	-0.01	-0.05	0.03	-0.02	-0.01	0.64	-0.14	-0.01
6. Echinoderms	0.06	0.01	0.07	0.00	-0.01	-0.34	0.04	-0.25	0.00	-0.47	0.08	0.00	0.01	-0.01	0.01	0.00
7. Zooplankton	0.13	0.18	0.26	-0.13	0.02	-0.04	-0.25	-0.03	0.00	0.02	-0.09	0.00	0.00	0.02	0.18	0.00
8. Polychaetes	0.03	-0.08	-0.04	0.04	-0.03	-0.06	-0.28	-0.43	0.00	0.05	-0.36	0.00	-0.35	-0.03	-0.08	0.00
9. Farmed Fish	-0.01	-0.03	0.00	-0.09	0.01	0.00	0.00	0.00	-0.63	0.00	0.00	-0.32	0.00	0.01	-0.03	0.37
10. Seaweed	0.00	-0.02	-0.04	0.05	0.10	0.07	-0.01	-0.04	0.00	-0.08	0.02	0.00	0.00	0.10	-0.02	0.00
11. Phytoplankton	0.14	0.10	0.20	-0.07	0.02	0.10	0.46	0.24	0.00	-0.08	-0.25	0.00	-0.20	0.02	0.10	0.00
12. Artificial Feed	0.03	0.07	0.00	0.21	-0.01	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	-0.01	0.07	0.29
13. Detritus	0.04	-0.04	-0.03	0.12	0.16	0.14	-0.04	0.14	0.00	-0.14	-0.11	-0.01	0.00	0.16	-0.04	0.00
14. Crustacean Fisheries	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15. Recreational Fisheries	-0.02	-0.22	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.22	0.00
16. Farmed Fish Harvesting	0.01	0.03	0.00	0.09	-0.01	0.00	0.00	0.00	-0.37	0.00	0.00	0.32	0.00	-0.01	0.03	-0.37

Table 8.8 Mixed trophic impacts for functional groups of Loch Melfort ecosystem in scenario 3. An impact of a group is represented by a number in the table. Numbers in rows represent impacts of the impacting group and those in the columns are the impacted groups. Positive/negative values represent an increase/decrease in the biomass of the impacting group and the corresponding increase/decrease of the biomass of the impacted group.

Impacting/Impacted	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Seabirds	-0.06	-0.49	0.00	-0.12	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	-0.49	0.00
2. Mackerel	0.06	-0.28	-0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00
3. Other Fishes	0.34	0.24	-0.14	-0.82	-0.09	0.00	-0.14	0.01	0.02	0.03	0.01	0.01	-0.09	0.24	0.02
4. Juvenile whiting	0.07	-0.08	-0.07	0.00	-0.06	0.00	-0.08	0.00	0.01	0.02	0.01	0.00	-0.06	-0.08	0.01
5. Crustaceans	-0.10	-0.15	-0.34	0.30	-0.35	-0.19	-0.11	0.01	0.00	-0.05	0.03	0.00	0.65	-0.15	0.00
6. Echinoderms	0.07	0.01	0.07	0.02	-0.01	-0.33	0.04	-0.24	-0.13	-0.47	0.09	0.01	-0.01	0.01	-0.13
7. Zooplankton	0.17	0.23	0.28	0.11	-0.01	-0.04	-0.26	-0.03	-0.04	0.03	-0.08	0.00	-0.01	0.23	-0.04
8. Polychaetes	0.03	-0.08	-0.02	-0.03	-0.02	-0.05	-0.24	-0.40	-0.19	0.04	-0.28	-0.36	-0.02	-0.08	-0.19
9. Farmed Mussels	-0.01	-0.02	-0.02	-0.01	0.00	-0.01	-0.05	-0.03	-0.35	0.01	-0.08	0.00	0.00	-0.02	0.65
10. Seaweed	0.00	-0.02	-0.04	0.05	0.10	0.07	-0.01	-0.03	-0.02	-0.08	0.02	0.00	0.10	-0.02	-0.02
11. Phytoplankton	0.16	0.12	0.21	0.08	0.01	0.10	0.44	0.24	0.41	-0.08	-0.28	-0.20	0.01	0.12	0.41
12. Detritus	0.03	-0.04	-0.04	0.09	0.16	0.14	-0.01	0.14	-0.05	-0.14	-0.09	0.00	0.16	-0.04	-0.05
13. Crustacean Fisheries	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14. Recreational Fisheries	-0.02	-0.22	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.22	0.00
15. Farmed Mussels Harvesting	0.01	0.01	0.01	0.00	0.00	0.00	0.02	0.01	-0.29	0.00	0.03	0.00	0.00	0.01	-0.29

Table 8.9 Mixed trophic impacts for functional groups of Loch Melfort ecosystem in scenario 4. An impact of a group is represented by a number in the table. Numbers in rows represent impacts of the impacting group and those in the columns are the impacted groups. Positive/negative values represent an increase/decrease in the biomass of the impacting group and the corresponding increase/decrease of the biomass of the impacted group.

Impacting/Impacted	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Seabirds	-0.06	-0.49	0.00	-0.11	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	-0.49
2. Mackerel	0.06	-0.28	-0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72
3. Other Fishes	0.35	0.24	-0.13	-0.83	-0.09	0.00	-0.13	0.01	0.02	0.02	0.01	-0.09	0.24
4. Juvenile whiting	0.07	-0.08	-0.07	0.00	-0.06	0.00	-0.07	0.00	0.01	0.01	0.00	-0.06	-0.08
5. Crustaceans	-0.10	-0.16	-0.35	0.30	-0.35	-0.19	-0.12	0.01	-0.04	0.03	-0.01	0.65	-0.16
6. Echinoderms	0.07	0.01	0.07	0.02	0.00	-0.34	0.04	-0.25	-0.48	0.08	0.02	0.00	0.01
7. Zooplankton	0.17	0.23	0.28	0.11	0.00	-0.04	-0.26	-0.03	0.03	-0.09	0.00	0.00	0.23
8. Polychaetes	0.02	-0.09	-0.04	-0.04	-0.02	-0.05	-0.28	-0.43	0.05	-0.35	-0.36	-0.02	-0.09
9. Seaweed	-0.01	-0.02	-0.04	0.05	0.10	0.07	-0.01	-0.03	-0.08	0.02	0.00	0.10	-0.02
10. Phytoplankton	0.16	0.13	0.21	0.08	0.01	0.11	0.45	0.25	-0.08	-0.26	-0.21	0.01	0.13
11. Detritus	0.02	-0.04	-0.04	0.08	0.15	0.13	-0.02	0.13	-0.14	-0.11	0.00	0.15	-0.04
12. Crustacean Fisheries	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13. Recreational Fisheries	-0.02	-0.22	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.22

8.4 Discussion

The ecosystem-based modelling approach taken in this study was useful in providing an insight into the general food web of Loch Melfort and how the food web may be affected by the presence of fish and mussel farming. Fish farming provides an additional food resource to the system. Mussel farming depends on the natural food available in the system. Both activities have direct and indirect impacts on different organisms. Using the the chosen set of parameters and functional groups the model provides one possible interpretation of the system with and without aquaculture activities. The model identifies areas that require further data collection to improve further development of the model.

8.4.1 The food web in Loch Melfort

Empirical data for a number of the groups included in the model was limited and thus a number of parameters were estimated by the model and based on the literature and/or other models. Thus, there was an increased uncertainty and the reliability of the model output. Despite some of the limitations in the model building and the potential underestimation/overestimation of parameters the model presents a minimal model of the food web in Loch Melfort.

Seabirds occupy the top trophic level in the system. Marine mammals, seals and porpoises, were noted during the fieldwork near the sea cages (see Chapter 5). There are also anecdotal accounts of both harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) on a nearby Isle of Shuna (Scottish Sanitary Survey Report 2015). However, marine mammals were not included in the model because of limited knowledge on the numbers visiting the loch and the duration of their visits. During the fieldwork a common skate was caught and released (see Appendix A). It is worth noting that the common skate is critically endangered by the International Union for the Conservation of Nature (IUCN) (Dulvy et al. 2006). The area from the Sound of Jura to Loch Sunart, including Loch Melfort, is a highly residential area for common skate and is a designated Marine Protected Area (Scottish Natural Heritage 2014). As common skate show site fidelity (Wearmouth and Sims 2009) it is likely that some skate might be resident in the Loch. These skate might benefit from the presence of fish farms by feeding on waste feed and/or other prey. The common skate was not included in the model because the model

was an attempt at a more general model that could be of use in other lochs. But future model improvement should take into account the presence of common skate in the area.

Other marine organisms that were not included in the model were suspension feeders such as wild mussels and cockles. In Loch na Cille, a shallow inlet at the head of Loch Melfort, a small fishery for common cockles (*Cerastoderma edule*) can be found (Scottish Sanitary Survey Report 2013). Although this group was not included echinoderms and polychaetes were included in the model and were assumed to play a similar role.

Most groups were set to have an EE of 0.95 in the model which means that biomasses are consumed within the system mainly by predation and minimal fisheries for crustaceans and mackerel. The model predicted an EE of 0.0 for seabirds which is expected for top predators that are not predated upon (Heymans et al. 2016).

The overall biomasses in the model were lowest for the higher trophic levels (seabirds and fishes). The biomass of seabirds is likely to be underestimated because there are a number of small isles just outside Loch Melfort that also have high number of seabirds (e.g. Scottish Sanitary Survey Report 2015). As seabirds are mobile it is likely that they visit the loch.

The highest biomasses were estimated by the model for the benthic organisms (crustaceans, echinoderms and polychaetes). Polychaetes and echinoderms were the largest two groups by biomass from the benthic organisms. Although limited to few sampling points and only qualitative data, the macrobenthic sampling in this study also indicated polychaetes and echinoderms as the most abundant benthic organisms (see Appendix A). Mente et al. (2010) reported echinoderms and polychaetes as the most abundant benthic organisms in four sea lochs along the West Coast. The echinoderms in their study were more abundant than the polychaetes but the polychaetes were more diverse (Mente et al. 2010). Similarly, Glud et al. (2016) reported that more than 95% of the macrofauna abundance in Loch Etive was accounted for by echinoderms (ophiuroids) followed by polychaetes and a smaller percentage of bivalves. The total wet biomass of the macrofauna in Loch Etive was reported at about 272 g/m^2 (Glud et al. 2016). In the model for Loch Melfort, the total biomass estimated for the benthic organisms was about 65.0 tonnes/km^2. Although these are two different areas and empirical data on the benthic organisms is needed in Loch Melfort, the higher P/B values used in the model may result in lower biomasses. The P/B values reported for benthic organisms (polychaetes, echinoderms, molluscs and crustaceans) in a high latitude fjord were below 1/year and in

some areas in the United Kingdom the P/B values for benthic organisms ranged from 0.4 to 1.28/year (Nilsen et al. 2006 and references therein). Nevertheless, P/B values used in the model for the benthic organisms are within range for benthic organisms in the Northern Atlantic (Cusson and Bourget 2005; Nilsen et al. 2006 and references therein).

The biomass of the macroalgae was estimated at 20.8 tonnes/km^2 which is more than the phytoplankton biomass entered in the model. Data on seaweed was lacking for the area but it has been suggested that macroalgae contribute significantly to primary production in coastal areas and play an important role in detrital and filter feeding food chains (Johnston et al.1977).

Loch Melfort receives organic carbon from terrestrial and phytoplankton flow. There is currently no study on the carbon budget of Loch Melfort. Loh et al. (2010) reported that the carbon input in Loch Creran is mainly from terrestrial sources followed by phytoplankton and a smaller portion of unknown sources (e.g. macroalgae). About 42.7% of the organic carbon entering the system is buried in the sediment, 48% is oxidised in the water and 19.3% is exported out of the Loch (Loh et al. 2010). Out of the terrestrial input, 63% were considered as labile and 37% as refractory organic matter (Loh et al. 2010). Overnell and Young (1995) reported the organic carbon budget for the upper Loch Lihne is also mainly from terrestrial sources and followed by phytoplankton. The authors noted that about 80% of the organic material in the loch is resuspended. The catchment area for both Loch Creran and upper Loch Linnhe are higher than that for Loch Melfort (Edwards and Sharples 1986). The Loch Melfort catchment area is smaller than for both of these lochs therefore is expected that the terrrestial inputs would be lower than in these two lochs. Nevertheless, the terrestrial input in the loch was assumed constant in all models and was not included.

The bacterial component associated with the degradation of organic material may play a significant role in the food web of the loch. Pedersen et al. (2016), using an Ecopath modelling approach, noted that the pelagic microbial food web was important in linking carbon from detritus to higher trophic levels in two fjords in Norway.

The overall ecosystem indices indicate that the system is productive and well connected. The PP/R of the system is 1.110 and falls within the range of PP/R between 0.8 and 3.2 reported in 41 aquatic systems (Christensen and Pauly 1993). The PP/R of the system is also close to the PP/R of 1 reported in two fjordic systems (Pedersen et al. 2016). Additionally, the PP/R ratio is close to the PP/R of 1.17 in marine coastal areas (Duarte and Agustí 1998). The PP/R ratio indicates that the system relies on primary

productivity slightly more than organic input in the system (Odum 1969). Odum (1969) indicated that systems are considered mature when PP/R ratio is close to 1. It is worth noting that the phytoplankton biomass in the model has not been estimated and is based on literature values for other lochs. As mentioned in Chapters 4 and 5, there is potential for phytoplankton growth within Loch Melfort because the flushing time is about 9 days which may give time for phytoplankton to grow. Additionally, the model does not include the bacterial biomass which may affect the PP/R ratio.

The higher primary productivity is also reflected in the primary production to total biomass ratio of the system. This index also indicates that if the primary production is higher than biomass the system may be in developing state whereas biomass increases in mature systems (Christensen 1995). Another two indices of maturity are the connectance and omnivory indices. Food chains become more web-like as a system becomes more mature (Odum 1969). The connectance index of the system was high (0.390) which indicates high diversity and relative stability. The connectance index was lower than the connectance index (0.154-0.168) reported for two fjords in Norway whereas the omnivory index (0.168) was similar to the omnivory index (0.178-0.183) in the two fjords (Pedersen et al. 2016).

Cycling of material in the system can give insight into the ecosystem functioning (Odum 1969). If energy is cycled through shorter cycles then the cycling is faster whereas if the energy is cycled through longer paths then the cycling is slower (Baird and Ulanowicz 1989). System that are more organised and recycle more are also considered more mature (Odum 1971). The Finn's cycling index and Finn's mean path length for the Loch Melfort system were 10.55% and 2.968, respectively. The Finn's cycling index in the system was slightly lower than the Finn's cycling index (15-17.9%) in two fjords in Norway (Pedersen et al. 2016). The Finn's mean path length in the Loch Melfort system was also lower than the Finn's mean path length reported for two fjords in Norway (3.87-4.18) (Pedersen et al. 2016). In a comparative study of four estuaries, including one on the east coast of Scotland, the Finn's cycling index ranged between 25-44% and the Finn's mean path length ranged between 2.86 and 3.95 (Baird and Ulanowicz 1993). The low Finn's cycling index and relatively short cycling paths suggests the system is in low level of maturity.

Overall, the MTI analysis indicated that predators have a direct negative effect on their prey and indirect negative effect on other groups that they share same resources with. Phytoplankton had a positive effect on almost all groups and zooplankton on the

top predators in the system (seabirds and fish). The analysis suggests that overall the lower trophic levels dominate the energy dynamics of the system.

8.4.2 Effects of aquaculture activities on the ecosystem

8.4.2.1 Effects of mussel farming

Cultured mussels feed on natural food particles in the water and produce faeces and pseudofaeces that contribute to the detrital pool (reviewed by Wilding 2011). Mussel farming can also affect the environment by the presence of the supporting structures, their living shells, and the dead shells that fall on the seabed (Wilding 2011; Wilding and Nickell 2013).

The biomasses of the groups 'other fishes', juvenile whiting, crustaceans, echinoderms, zooplankton, polychaetes and seaweed decreased when mussel farming was added to the system. The cultured mussels have the potential to indirectly affect mackerel, juvenile whiting and other fishes by competing with zooplankton for phytoplankton. Mussels have the potential to compete with zooplankton particularly in temperate waters (see review by Wilding 2011). Polychaetes were also set to consume higher proportion of phytoplankton which also leads to competition with cultured mussels for phytoplankton.

Lin et al. (2009) used an Ecopath with Ecosim modelling approach to predict the biomass changes of a number of functional groups after the removal of cultured oysters in an eutrophic poorly flushed lagoon in Taiwan. The researchers noted that the biomasses of phytoplankton, zooplankton, and detrivorous fish increased following the removal of oyster culture. This was similar to the patterns detected in the Loch Melfort ecosystem with mussel farming. However, Lin et al. (2009) detected decrease in benthic organisms which they related to the decrease in biodeposition from the cultured oysters. This was contrary to the patterns detected in this study which maybe because the biodeposits from the farmed mussels were set at a minimal proportion in the diet of all organisms. Lin et al. (2009) also noted that there was an increase in biomass of some fish and a major decrease in biomass of other fish after the removal of the oyster culture. The decrease of biomass in some fish was related to the artificial habitat that the oyster provide for some fish (Lin et al. 2009). The effects of the physical contribution of the mussel farming to the Loch Melfort system is not captured using the Ecopath model. Kluger et al. (2016) also reported that the presence of large quanitites of scallops

(*Argopecten purpuratus*) in Sechura Bay (North Peru) resulted in increase in biomasses of some predators and decrease in biomasses of their competitors.

The addition of cultured mussels in the system was also noted in the overall change in parameters of the system. Some decrease was noted in the total system throughput, total biomass, connectance and omnivory indices, Finn's cycling index and mean's path length whereas some increase was noted in the PP/R ratio, net system production and PP/B ratio. Similar high PP/R, PP/B, low omnivory index, low Finn's cycling index and path length were also reported for a highly productive tidal bay in France (Table 8.5; Leloup et al. 2008).

If mussel culture replaces zooplankton in the ecosystem the food web can be reduced to the presence of lower trophic levels (nutrient, phytoplankton, farmed mussel, detritus) with no high trophic levels (Jiang and Gibbs 2005). Although mussel farming has the potential to compete with zooplankton the effect is very minimal in this model. The mussels were also set to feed mainly on phytoplankton but other food sources need to be considered such as heterotrophs (see review by Wilding 2011). Further modelling is needed to establish the ecological capacity or "the stocking or farm density which causes unacceptable ecological impacts" (Inglis et al. 2002) of the system. Byron et al. (2011), using the Ecopath modelling approach, reported that oysters cultured in a highly flushed and productive temperate lagoon with a biomass of 12 tonnes/km^2 live weight need to increase in biomass by 62 times in order to exceed an ecological carrying capacity of 722 tonnes/km^2. In a more oligotrophic bay in New Zealand, Jiang and Gibbs (2005), using Ecopath modelling approach, reported an ecological carrying capacity of 65 tonnes/km^2. In the present model, the estimated biomass for the cultured mussels was only 4.950 tonnes/km^2 which is possibly much lower than the ecological carrying capacity of the system.

8.4.2.2 Effects of fish farming

Fish farming adds particulate organic waste (waste feed and fish faeces) to the system which was detected in the increased biomasses of the groups 'other fishes', juvenile whiting, crustaceans, echinoderms, zooplankton, polychaetes, and seaweed (scenario 2). Similarly, Díaz López et al. (2008), using Ecopath modelling approach, noted a substantial increase in the biomasses of different functional groups and the overall total biomass following the addition of fish farming in Aranci bay, Sardinia Island (Italy)

(Table 8.5). Although no increase in the biomass of mackerel was detected the observations and results of the fieldwork in 2013 and 2014 (Chapters 4 and 5) indicated an overall increase in the biomass of marine organisms around the sea cages. It is also worth noting that model comparison of different systems is difficult because there are differences in study areas, study protocols and the selection of functional groups.

The MTI analysis indicated that increase in the artificial feed biomass would have a positive effect on mackerel and a stronger positive effect on whiting. Artificial feed would also have indirect positive effect on seabirds via the increase in fish biomass. However, slight increase in phytoplankton would have indirect negative effects on whiting which is through the increase in the biomass of other fishes. There is more phytoplankton accumulating in the system because of the increased organic input.

The overall parameters of the system were also affected by the organic input in the system. The PP/R, PP/B, connectance index decreased whereas the omnivory index, Finn's cycling index and Finns'mean path length slightly increased as compared to scenario 4. Similar decrease in PP/R, PP/B was reported following the addition of fish farming in Aranci bay, Sardinia Island (Table 8.5). Low PP/R, PP/B, and connectance index were also reported for a fish farming area, Santa Pola Bay, Southwestern Mediterranean Sea (Spain) (Table 8.5; Bayle-Sempere et al. 2013). The overall system was considered immature however the addition of organic input into the system from fish farming provided the system with greater resilience to perturbations (Bayle-Sempere et al. 2013). Bayle-Sempere et al. (2013) noted that the system was less dependent on primary production and the presence of wild fish around the cages reduced the build-up of nutrients. Results from Chapters 4 and 5 indicated that some fish around the sea cages consume the waste feed but other organisms are also likely to benefit from the additional food resources that the farm provided.

8.4.2.3 Effects of both activities

Some decrease in the biomasses of other fishes, juvenile whiting, crustaceans, echinoderms, polychaetes, zooplankton and seaweed were noted when both aquaculture activities were present in the ecosystem. The overall biomass of the system is between that of scenarios 2 and 3. The presence of fish farming added particulate organic input in the system and increased the bioaccumulation of phytoplankton whereas the mussel

farming depends mainly on the phytoplankton in the system. The system parameters were between scenario 2 and 3.

In the presence of both activities the farmed mussels had slight negative effect on mackerel and other fish groups and slight positive effects were detected for juvenile whiting whereas when only farmed mussels are present in the system there is a slight negative effect on mackerel and other fishes but slight positive effect on whiting. This is because when both activities are present the nutrient loading reduces some of the pressure on zooplankton. Additionally, the increase in phytoplankton would increase zooplankton biomass which are main food item for the group other fishes which in turn predate on juvenile whiting.

Although both aquaculture activities can have different effects on the ecosystem it is worth noting that the overall change induced in the system is not very large. The system is also assumed to be highly productive and impacts on the system may not be as obvious if the system were oligotrophic (e.g. Bayle-Sempere et al. 2013). It is worth noting that the level of impact of both aquaculture activities on the various functional groups was set at minimum. Nevertheless, as Goodbrand et al. (2013) noted that even in high productive areas, marine aquaculture can induce ecosystem-level effects.

The model can be improved and adapted to other sea lochs. Previous models by Haggan and Pitcher (2005) and Alexander et al. (2015) described the food web on the West Coast; however no aquaculture activities were included in the models. As the West Coast hosts many fish and mussel farms it would be of interest to see how the ecosystem is affected by their presence.

Impacts of aquaculture activities can be positive, negative or none depending on the species and aquaculture activity.

8.4.3 Model assumptions and limitations

The modelled scenarios provided static snapshots of the Loch Melfort ecosystem in 2013/2014 with and without aquaculture activities. However, knowledge of Loch Melfort ecosystem in general is limited and even more limited for the studied period. The model scenario with aquaculture activities includes only 14 functional groups and other groups can be included as discussed in subsection 8.4.1. This is mainly because of the lack of data such as biomass and diet composition for most of the functional groups in Loch Melfort. Some parameters were based on the literature, estimated by the model, and

based on other models (e.g. Haggan and Pitcher 2005; Alexander et al. 2015). Estimating parameters for the model with accuracy affects the output of the model. Parameter estimates for aggregated groups (e.g. other fishes, crustaceans, echinoderms, polychaetes) are almost impossible to estimate with accuracy as these groups include a lot more species than could possibly be included in the model. Dietary composition of various groups and/or individual species had to be based on estimates from the combination of various diets or similar species (Jiang and Gibbs 2005). Temporal changes in diet have not been considered.

The models are only descriptive and cannot predict any future patterns. However, as knowledge of the system improves and more data is collected other parts can be added such as temporal and spatial components.

For the purpose of Ecopath modelling, the studied area is assumed closed and there is no consideration of migration patterns of seabirds and fishes between the sea loch area and the wider sea beyond. As noted by the models of Ross et al. (1993) and (1994) the import and export of nutrients is important in the sea loch system and phytoplankton growth is regulated by light, temperature and higher trophic levels. These aspects were not taken account of in the present model. The aim of the modelling approach in this study was to compare a loch system with and without aquaculture activities, assuming that the export and import is equal. To capture the export and import of nutrients another ecosystem modelling approach taking account of the hydrodynamics in the loch may be useful.

Another limitation of the model is that the whole system is assumed to be affected by the presence of the aquaculture activities. The waste from both aquaculture activities are assumed to affect the entire loch and do not take into account the localised nature of the impact.

Although ecosystem models allow the addition of a number of species and capture various processes the increased realism requires more data as compared to single-species models (Fulton et al. 2003; Latour et al. 2003). Additionally, ecosystem based models suffer from other issues such as determining what functional groups and processes need to be included in the model, defining the indices to summarise model outputs (Fulton et al. 2003). Fulton et al. (2003) recommended the use of several simpler models rather than using one complicated ecosystem model as too much complexity leads to uncertainty and difficulties in interpreting the model. The use of several simpler models may be of use in future modelling of the system.

8.5 Conclusions

Fish farming has an impact on the food web via the nutrient loading whereas the mussel farming relies on the natural food in the system. Both activities have the potential to induce direct and indirect effects in the system. Fish farming decreases the reliance on primary productivity in the system whereas mussel farming can compete with zooplankton for resources which affect higher trophic levels. The combination of both fish farming and mussel farming has an overall potential to reduce some of the effects that each of these activities can induce if present on their own.

The present model is only a guess of the food web in the Loch Melfort ecosystem. The ecosystem-based approach undertaken in this study is a useful tool in describing the impacts of aquaculture activities on the food chain and evaluating different hypothetical situations. Moreover, the models also identified knowledge gaps about the Loch Melfort ecosystem. To improve the models further fieldwork studies are needed to obtain information on biomasses, production, trophic-links between groups (predator-prey relationships).

PART III: GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 9
COMBINING EMPIRICAL AND MODELLING STUDIES REVEALS A MORE HOLISTIC VIEW OF AQUACULTURE EFFECTS

9.1 Introduction

In order to understand the ecological consequences of aquaculture presence in marine environments this thesis evaluated the direct and indirect ecological effects of coastal aquaculture activities on wild marine non-salmonid fishes sampled near two fish farms on the West Coast of Scotland. Empirical approaches were used to establish the direct impacts of fish farming at the individual level of wild fish caught near sea cages (Chapters 4, 5, and 6). Modelling approaches were used to extrapolate the direct effects detected at the individual level to population (see Chapter 7) and ecosystem (see Chapter 8) levels. Indirect effects are also detected using the ecosystem modelling approach (Chapter 8).

In these conclusions, I discuss results of the empirical and modelling studies and draw overall conclusions about the types and magnitudes of the effects of Scottish marine cage aquaculture on wild fish communities. Based on the main findings of each chapter I draw lessons on how both empirical and modelling approaches are needed to understand new ecological interactions with limited observations. I summarise the results of Chapters 4-8 in sections 9.2 to 9.4. The potential implications for fisheries, conservation and the environment are described in section 9.5. The limitations and improvements in using both empirical and modelling approached are described in section 9.6. Conclusions of the thesis can be found in section 9.7.

9.2 Fish farms attract fish and lead to direct individual level impacts

In order to understand whether coastal sea cages create new habitats for marine fish communities in lochs and whether the benefits are positive, negative or none it is essential to establish whether artificial structures only locally attract (redistribute) fish, increase productivity (via increased growth, survival, reproduction) (Bohnsack 1989; Lindberg

1997; Pickering and Whitmarsh 1997) or provide poor habitats (ecological traps) that lead to an overall reduced fitness (survival, reproduction) (Reubens et al. 2014).

The observations during fieldwork of 2013 and 2014 (Chapters 4 and 5; detailed in Appendix A) indicated that various fishes were attracted to coastal sea cages and this is consistent with other reports worldwide (see Chapter 1; Sanchez-Jerez et al. 2011; Uglem et al. 2014). Results in Chapter 6 indicated that fish of different sizes are attracted to fish farms. For mackerel there was wide variation in lengths whereas for gadoids the length range was restricted. This is consistent with the behaviour of mackerel where fish of all lengths visit the West coast to feed and of gadoids that use the lochs as nursery ground (Lockwood 1988; Ware 2009). Sampling methodology can affect the length distribution of fishes (Løkkeborg and Bjordal 1992).

Although increased presence of fish around the cages was observed, I did not quantify catch per unit effort (CPUE) near and away from cages. Sampling bias is likely because technique and experience improved catchability over time and it is subject to high level of stochastic variation. Additionally, catchability was not controlled with environmental conditions (low and high tides, sunny/rainy weather). In addition to catch data visual observations using underwater video equipment or diver based techniques need to be undertaken to estimate abundance and observe behaviour of fish near and away from the sea cages. I used underwater video recordings to observe fish around fish farms; however bad weather conditions did not allow consistent collection of recordings near and away from fish farms. Therefore, abundance and biomass of wild fish around fish farms was not estimated.

9.2.1 Coastal sea cages provide enhanced feeding (natural and artificial food) grounds for fish

In general, the addition of nutrients and detritus in a habitat increases primary productivity and the abundance of prey organisms (reviewed by Polis et al. 1997). Eveleigh et al. (2007) termed the increase of mobile predator density in response to increase in natural resources as the “birdfeeder effect”. Similar response has been noted for mobile organisms and fish farms.

Fish farming releases dissolved organic and inorganic nutrients, particulate organic matter (waste feed and faeces) (Olsen et al. 2008). Dissolved nutrients released from fish farming (review by Holmer 2010; Price et al. 2015) have the potential to stimulate the

growth of phytoplankton (Islam 2005) which can be a source of food for higher trophic levels. Dissolved nutrients from the fish farms can also be taken up by bacteria and macroalgae in the loch systems (reviewed by Olsen et al. 2008). This can also increase the food resources for different organisms in the system. It is also worth noting that high nutrient input will increase the inflow of dead organic matter to the sediment (Olsen et al. 2008).

In this study, the salmon farm was located in a highly flushed sea loch and the potential for *in situ* phytoplankton growth is minimal (flushing time = 3 days (Chapter 3); phytoplankton growth 3-5 days (Olsen et al. 2008)). The halibut farm was located in a less flushed sea loch (flushing time=9 days (Chapter 3)) and the potential for *in situ* phytoplankton growth is higher than that for Loch Leven. In a recent study on the nitrogen dynamics and phytoplankton structure in Loch Creran, Moschonas et al. (2017) reported that organic nitrogen input from anthropogenic activities may contribute to local production. In general, in Scotland no consistency has been found between fish farm nutrient release and primary productivity (see Price et al. 2015). It is also worth noting that in general there is poor understanding of how nutrients from fish farms and other anthropogenic sources affect the pelagic system (reviewed by Olsen et al. 2008). In this research, I mainly focused on detecting the particulate organic matter from the two fish farms and further studies may be useful.

Particulate organic matter discharged from the sea cages is in the form of waste feed and faeces (e.g. Holmer 2010; Price et al. 2015). There is limited information on whether wild fish communities benefit from the additional food resources released from fish farms in Scotland. Previous studies reported saithe as the main species to have consumed waste feed from sea cages in Scotland (Carss 1990; Mente et al. 2008). In this study (Chapters 4 and 5), using stomach content analysis, I found mainly mackerel and whiting to have consumed waste feed. Although, only few saithe were sampled near the sea cages waste feed was found in some of these fish (Chapter 4). Other fish species, including saithe, have been found eating on waste pellets from coastal fish farms in other countries (reviewed by Uglem et al. 2014; Chapter 1). The consumption of particulate organic matter by various marine organisms in Loch Melfort was also incorporated in the ecosystem models (Chapter 8).

Although stomach content analysis is a useful tool in gaining understanding into the diet of a species it only reveals the most recently ingested meal by the fish (see Chapter 2). Fatty acids have been used as biomarkers to detect the fish farming influence

in Norway and the Meditteranean Sea (e.g. Skog et al. 2003; Fernandez-Jover et al. 2007a, 2009, 2011a; Arechavala-Lopez et al. 2015a). I used fatty acid biomarkers to detect the influence of two fish farms on mackerel, saithe and whiting sampled near the sea cages (Chapters 4 and 5). Waste feed (or faeces) consumption by mackerel, saithe and whiting around the sea cages was detected in the modified FA profiles of their muscle tissues (Chapter 4 and 5). Both muscle and liver tissues were useful in detecting modifications in FA profiles in mackerel and saithe sampled near a halibut farm (Chapter 4). Mackerel, saithe and whiting sampled at both the halibut and salmon farms had elevated levels of 18:2n-6 which is indicative of vegetable oils in the diet (Chapter 4-5). Mackerel and whiting sampled near the salmon farm also had elevated levels of the 18:3n-3 FA indicator of vegetable oils in the diet (Chapter 5). The impacts of the salmon farm on mackerel and whiting FA profiles appeared to be stonger than the impacts of the halibut farm on the FA profiles for both species (Chapter 5). The reason for this is the higher replacement of vegetable oils in the salmon feed. It is worth noting that the diets change from year to year and also depend on the cultured species and stage of production (see Chapters 4 and 5). Hence, the use of 18:2n-6 and 18-3n-3 as biomarkers for fish farming impacts on wild fishes may not be always reliable in the long term and other biomarkers need to be explored.

The increase in prey around the sea cages was also noted in the underwater recordings and the stomach content analysis of mackerel, whiting and saithe (Chapters 4-6; Appendix A; this was also considered in Chapter 8). In Loch Melfort, mackerel schools were preying upon the clupeids. Although only based on anecdotal accounts seals and porpoises were noted when schools of mackerel were chasing after schools of clupeids around the sea cages in Loch Melfort. In Loch Leven, bigger predators such as thornback, dogfish and seals were also noted around the sea cages.

Besides the provision of nutrients from the fish farms the sea cages provide physical structure in the water (see Chapter 1). The structures (e.g. cages, nets, floats, ropes) that make up a fish farm provide surfaces for animal, plants and microbes also known as biofouling (Fitridge et al. 2012). Artificial structures initially become colonized by biofilms (aggregates of mucus, microalgae, and bacteria) which in turn become source of food for grazers (e.g. echinoderms and gastropods) and subsequently food for higher trophic levels (see Tan et al. 2015 and references therein). In the underwater video recordings taken during the fieldtrips of 2013/2014 (Chapter 3) to the two fish farms, a number of organisms were noted on the sea cage structures (see Appendix A) which are

likely to be source of food for higher trophic levels. It is also worth noting that bifouling is damaging to the aquaculture industry and a number of measures are taken to reduce biofouling including net changing and cleaning, use of chemical antifoulants and the use of biological control (Fitridge et al. 2012). The cleaning of the nets would lead to increased organic matter that would contribute to the detrital pool.

Complexity of structures also appears to be important for productivity (Langhamer 2012). Langhamer (2012) noted that juvenile organisms benefit from natural habitats such as coral reefs, mangroves and sea grasses not only for their high productivity but also for the highly complex substratum that provides niches of different sizes as shelter for different organisms. Juvenile whiting avoids predators by using highly complex habitats such as eelgrass, rocky habitats macroalgae and reef habitat as shelter (Bailey et al. 2011). Sea cages have the potential to provide similar artificial habitat that can be used by young fish as shelter. As in artificial reefs the effects of aquaculture on marine fish communities depend on the location of aquaculture activities and the characteristics of the local populations (Langhamer 2012).

9.2.2 More food: better condition

In general, the presence of an easily accessible anthropogenic resource subsidies in a habitat often leads to improved physiology of animals that exploit these resources (reviewed by Oro et al. 2013). Wild fish consuming high energy waste feed and natural food items near sea cages often have higher body fat and improved condition indices such as Fulton's condition index (FCI) and hepatosomatic index (HSI) (see Sanchez-Jerez et al. 2011; Dempster et al. 2011; Chapter 4-6). No differences in FCI and HSI were noted in mackerel sampled near and away from sea cages (Chapter 4 and 5). However, when the data were pooled across all sites and years, some differences in FCI were detected between both groups of mackerel (Chapter 6). For the gadoids overall improvement in FCI was noted in saithe sampled near sea cages as compared to fish from reference sites and no statistically significant differences in condition indices were noted for whiting (Chapters 4 and 6). Improved condition indices can indicate a higher reproductive output (Sanchez-Jerez et al. 2011; see also discussions in Chapters 4-5).

9.3 Fish farms: population sources, ecological traps or none?

The availability of resource subsidies in the environment has the potential to increase population growth rates, abundance and size of organisms that take advantage of such resources (reviewed by Oro et al. 2013). Oro et al. (2013) also noted that the introduction of food resources in the environment can also create ecological traps with the potential to decrease the population growth rates.

Based on empirical evidence collected during 2013 and 2014 (Chapters 4-6), the condition of fish sampled near two fish farms were overall better than those sampled away from sea cages. This indicates the potential for local production and increased biomass of fish. Although condition was improved for some fish feeding around the cages, results from Chapters 4 and 5 also indicated that fatty acid profiles of mackerel, juvenile whiting and saithe are modified to reflect the diet of the waste feed. It is not clear what the impacts of such modifications are on the egg quality and larvae survival of wild fish (see Sanchez-Jerez et al. 2011). However, it is worth noting that feed ingredients such as fish oil and fish meal containing high levels of n-3 PUFAs (20:5n-3 and 22:6n-3) are limited and expensive and therefore there has been increasing research efforts to find alternative replacements such as using plant-based ingredients (Tacon and Metian 2008). Other potential alternatives for terrestrial based feeds for fish meal and fish oil include microalgae (Sprague et al. 2016) or genetically modified oilseed crop plants that can synthetize n-3 PUFAs (Betancor et al. 2015). Changes in FA profiles of wild fish feeding waste feed will be minimal as ingredients in the fish feed change towards ingredient that are similar to the natural feed of fish.

Other potential negative impacts of fish farming on wild fish include elevated levels of predation, presence of fishing industry, anglers, transfer of diseases, and elevated levels of contaminants (see Chapter 1). Oro et al. (2013) noted that hyperpredation can occur as a result of resource subsidies which can change the predator-prey relationships. High predation rates were noted during some of the sampling trips around the sea cages. For example, mackerel was recorded predating on schools of clupeids (Appendix A). However, the presence of waste pellets can potentially decrease predation on juvenile fish (Fernandez-Jover et al. 2009). Based on anecdotal information from fish farmers, juvenile fish (e.g. saithe) enter the sea cages through the nets and remain inside the cages until harvest. This has not been quantified and thus it is not clear to what extent such mortality affects the juvenile populations. Coastal fish farms can also act as an ecological

trap if potential contaminants from fish farming are transferred in the wild fish flesh which can potentially disrupt endocrine processes resulting in negative impacts on reproductive processes (Bustnes et al. 2010). Pollutants can have negative effects on reproductive success of inshore fish such as whiting populations (see Bailey et al. 2011 and references therein). However, a recent study by Lundebye et al. (2017) indicated that the level of contaminants are lower in farmed salmon than in wild salmon. It is also worth noting that the feed offered to farmed fish are more controllable and parasite free (e.g. Dempster et al. 2011).

The impacts of fish farming whether positive or negative can interact in complex and unpredictable ways. It is also important to assess whether fish farming effects on individuals at specific sites will produce strong enough effects at the population level of fish. The overall potential for the fish farms to act at the extremes as either population sources or ecological traps is higher for juvenile whiting than for mackerel (Chapter 7). Fish farming can be a population source for about 26% of the mackerel and 64% of the whiting populations visiting the sea cages. Based on a very rough estimates about 0.75% of the total mackerel biomass and 0.73% of the total whiting biomass on the West coast would be impacted by fish farming. The proportion of total saithe biomass affected by fish farming on the West coast is assumed to be similar to that of whiting. Although the proportions are similar for all species the impacts are stronger for whiting and saithe than for mackerel (Chapter 7). Based on the empirical evidence in Chapters 4-6 and literature (Chaper 1) there appears to be potential for the two fish farms to act as population sources. This is also consistent with results reported by Dempster et al. (2011) that fish farms act as population sources for saithe and cod.

9.4 Direct and indirect aquaculture effects on the ecosystem

The presence of natural or artificial resource subsidies in the environment can induce changes across the food web (see Polis et al. 1997; Oro et al. 2013). The addition of a resource can stimulate primary production with subsequent increase in higher trophic levels (Polis et al. 1997). If the natural subsidy is not a source but a consumer then the increased predation on the prey can release pressure on the next lower trophic level (Polis et al. 1997). Trophic cascades can also take place when the subsidy is of anthropogenic origin (e.g. Oro et al. 2013; Newsome et al. 2015). It is also worth noting that the presence

of physical structure can also alter trophic cascades (see Newsome et al. 2015 and references therein).

Goodbrand et al. (2013) noted that animals learn and exploit spatially and temporarily predictable resources (frequent and intense) such as artificial feed which is similar to the response of an animal to a resource pulse (infrequent, intense and short in duration) but with bigger impacts on the animal (Yang et al. 2008). Consumers of resources attract predators which at a high density pose a risk of pathogen transmission (Yang et al. 2008). Yang et al. (2008) noted that resource pulses can have broad impact on the ecosystem and even after a resource pulse is over the impacts can persist long term. Thus, predictable resources from fish farming are likely to have a long term effect on the ecosystem.

The ecosystem model presented in Chapter 8 was informed by the empirical data presented in Chapters 4-6. The models indicated that fish farming has an impact on the food web via nutrient loading (see also Díaz López et al. 2008; Bayle-Sempere et al. 2013) whereas mussel farming relies on natural food sources and has the potential to impact the food web via competing with zooplankton for resources which can affect higher trophic levels (see also Jian and Gibbs 2005). The presence of both activities can balance the impact when each activity is present on its own in the ecosystem. Both activities have the potential do induce direct and indirect effects in the system. It is worth noting that these are only hypothetical scenarios and further research is needed to verify or dismiss the parameters in the model and to overall improve the model outputs.

9.5 Potential implications for fisheries, conservation and environment

In 2014, the number of active sea fish farms was 260 (Munro and Wallace 2015a) located in about 111 sheltered sea lochs (Gillibrand et al. 2002). Using very rough estimates the total waste feed from sea cages is about 10, 740 tonnes and the total faecal matter is about 33, 000 tonnes, assuming 5% waste feed, an FCR of 1.2 and a 15% undigestibility (Chapter 1; Gillibrand et al. 2002). Both the addition of particulate organic matter and the presence of physical structure can directly and indirectly affect wild fishes. Both aquaculture activities and small scale commercial and recreational fishing take place in most sea lochs and thus it is expected to have ecological interactions between both sectors as they share the same resources. Ecological interactions between fish farming and offshore commercial fishing on the West Coast are more likely to be indirect.

9.5.1 Potential benefits of fish farming to fisheries and conservation

The benefits of fish farming will vary among fish species. Similar to marine reserves, coastal sea cages can benefit sedentary fish and species that spend more of their life around the cages. Mobile species can also benefit if for example a small portion of the population remains longer around fish cages which would enable the build-up of biomass and exportation to fishing grounds (e.g. Gell and Roberts 2003). As indicated from empirical and modelling approaches (Chapters 4-7) there is higher potential for the fish farms to act as population sources.

If the evidence collected near the two fish farms in this thesis is similar across all fish farms then the benefits in terms of biomass and/or reproductive capacity will be exported far beyond the sea cages boundaries which can have subsequent benefit to local commercial and recreational fishing industries. It is worth noting that mackerel is the most valuable pelagic stock in Scotland (Munro and Wallace 2015a) and any benefit from aquaculture on the stock would reflect positively on the fisheries industry.

Based on this study it is not evident whether there is an increased productivity of the species in terms of biomass at the regional level. Moreover, only a bit less than a 1% of the regional population is expected to visit the sea cages and to be directly impacted. Further studies need to assess whether there is an increase in regional production such as increase in total regional catch related to the presence of coastal sea cages. Difficulties in measuring production at a regional scale can also arise from change in other environmental factors that can mask the increase in production.

Anthropogenic impacts, such as fish farming, in nursery areas can affect juvenile fish abundance and subsequently affect the year-classes strength which can be traced back six years after the settlement year (Bailey et al. 2011). Thus, any fish farming impacts on the juvenile stages of commercially important fishes such as saithe and whiting can indirectly affect the offshore fishing industries.

Arechavala-Lopez et al. (2011) reported that local artisanal fisheries located in scales of kilometres from the nearest fish farm in the Mediterranean Sea benefitted from the fish farms by the export of wild fish biomass. However, the researchers did not find aquaculture influence on trawlers operating at a distance of tens of kilometres from the farms. Fish farming influence on local fisheries landings was also reported by Machias et al. (2006) and Izquierdo-Gomez et al. (2015).

Several studies have suggested that coastal sea cages can act as small marine protected areas (MPAs) provided there is no commercial or recreational activities near fish farms (e.g. Dempster et al. 2002, 2005, 2006; Özgül and Angel 2013). In this study, Loch Melfort is within the Loch Sunart to sound of Jura MPA (Scottish Natural Heritage 2014). As such endangered species such as the common skate can potentially find benefit from the fish farms in terms of protection and food.

9.5.2 Implications for the environment

The presence of wild fish around sea cages can reduce the amount of waste feed from the sea cages and subsequent effects on the benthos (see also Chapter 1; subsection 1.7.1). Some wild fish might be of benefit to the cultured fish by reducing external parasites. For example, Carss (1990) noted that saithe caught near and away from fish farms had sea lice (*Lepeophtheirus* sp.) in their diet, common external parasites in wild and farmed salmon, which could be linked to fish farming. During fieldwork of 2013 and 2014, the staff at both farms noted that young gadoids enter the sea cages and thus may feed on some of the sea lice. This information, however, is only anecdotal.

9.5.2.1 Environmental regulation of fish farming in Scotland

Olsen et al. (2008) noted if the environment is harmed and the water quality is inadequate the aquaculture industry is the first to suffer from the consequences. In Scotland, any new and existing aquaculture activity is regulated to assure that the environment is not harmed. Prior to any new aquaculture development in Scotland a number of licences need to be acquired from statutory consultees such as Marine Scotland Science (MSS)[9], Scottish Environment Protection Agency (SEPA) [10], Scottish Natural Heritage (SNH)[11] and Fisheries Management Scotland)[12]. Different organisations regulate different aspects of the establishment of a new aquaculture project. For example, SEPA is a government agency responsible for protection of the environment and activities such as aquaculture to promote the application of legislation (The Water

[9] http://www.gov.scot/Topics/marine/science [Accessed: 4 February 2018].
[10] http://www.sepa.org.uk/ [Accessed: 4 February 2018].
[11] http://www.snh.gov.uk/ [Accessed: 4 February 2018].
[12] http://fms.scot/ [Accessed: 4 February 2018].

Environment (Controlled Activities) (Scotland) Regulations 2011[13] and amendments[14] or commonly known as the Controlled Activities Regulations (CAR)). SNH is responsible for the conservation of Scottish environment and works in agreement with SEPA, MSS and FMS to ensure the proper marine aquaculture planning[15]. FMS is involved in protecting and improving salmon and seatrout in Scotland.

9.5.2.2 Potential management solutions

Better nutrient waste management and ecologically engineered fish farms could reduce some of the issues related to nutrient loading. Ecologically engineered fish farms can be designed in a way to increase structural complexity which can provide additional habitat for aquatic organisms around the sea cages that can potentially reduce the nutrient impacts to the surrounding environment (Costa-Pierce and Bridger 2002). Building artificial reefs around fish farms can also attract aquatic organisms that have diverse feeding habitats and can use the nutrient resources released from the farms. This can reduce the nutrient loading into the environment (Costa-Pierce and Bridger 2002; Jan et al. 2014). Integrated multitrophic aquaculture is another environmentally friendly solution for reducing the nutrient loading (e.g. Hughes and Black 2016).

9.6 The use of pluralistic approach

Fieldwork experiments and observations taken in the first part of the thesis aimed at studying the direct effects of two fish farms on wild fish sampled near the sea cages. However, such an approach is always limited due to logistics and the complexity of the environment. Models are a useful tool for researchers because they simplify a rather complex environment. Simplification of the generated models cannot capture all the components of the natural system at the same time (Jørgensen et al. 2016) and thus as Box (1976) noted "all models are wrong, but some are useful". A combination of empirical and modelling work was important to understand the ecological impacts of aquaculture activities on wild fish communities.

[13] http://www.legislation.gov.uk/ssi/2011/209/contents/made [Accessed: 4 February 2018].
[14] http://www.legislation.gov.uk/ssi/2013/176/contents/made [Accessed: 4 February 2018].
[15] http://www.gov.scot/Resource/Doc/295194/0106302.pdf [Accessed: 4 February 2018].

9.6.1 Empirical methodologies

No sampling design is ideal and this was the case in this thesis. As noted in Chapter 3, logistics restricted the number of sampling sites and samples that can be collected and analysed. Finding control sites was a challenge because there was a lack of sites with no fish farming similar to those with fish farming. As this study involved only two different fish farming sites extrapolating from these two sites to other sites should be made with caution as there is natural variability in the environment.

Using a static underwater video camera was very useful in capturing mackerel feeding waste feed from the sea cages and chasing after clupeids (Appendix A). However, it was difficult to operate beyond a certain depth because of strong currents and poor water visibility (Chapter 3). On one occasion the underwater video camera was baited and that appeared to be useful in recording organisms around the sea cages. However, this was a very brief trial and further trials are needed if it might be a useful technique. The use of hook and line was a cheap and efficient method to catch fish near the sea cages. However, the methodology was restricted by the fishing gear. Based on the methodology used mackerel and whiting were the most common fish sampled. Another potential molecular tool, alternative to fishing and visual observations, is using environmental DNA (eDNA) (Rees et al. 2014; Yamamoto et al. 2017). Lejzerowicz et al. (2015) used eDNA to described benthic communities near and away from a fish farm and advocated the use of eDNA in monitoring the quality of the benthos.

Both stomach content analysis and fatty acid analysis were useful tool in detecting the influence of two fish farms on the sampled wild fish. In particular, the fatty acid analysis was a good tool in detecting waste feed from both a halibut and a salmon farm. In order to trace waste feed in the food web other organisms could be sampled such as benthic organisms, pelagic and top predators. This would give a better understanding of aquaculture influence on the food web.

Various tagging techniques (e.g. electronic tags (Metcalfe and Arnold 1997)) could be used to trace the movement of different fish in relation to aquaculture activities. Tagging studies would also be useful in estimating what proportion of the regional population is affected by aquaculture activities. The use of tagging requires resources and cooperation between the aquaculture and fisheries sectors. Otoliths can be used as natural tags (reviewed by Gillanders 2005) and the otolith shapes can differentiate between different ecotypes of fish (e.g. Bardarson et al. 2017). Otolith microchemistry would be

highly useful in detecting aquaculture impacts on wild fish populations (e.g. Kalish 1987). The advantage of using otoliths as tags over the conventional tags is that these are are present throughout the life of a fish, can be related to the age of the fish and are permanent (Elsdon et al. 2008).

9.6.2 Modelling approaches

The univariate and multivariate statistical models applied to the empirical data collected during fieldwork in 2013 and 2014 were overall considered as useful approximation fits to the data. Using multivariate statistical models was a more powerful tool than univariate statistical models in distinguishing between fish sampled near and away from sea cages based on their FA profiles.

The Leslie population matrix models were very useful in building the phase space model in Chapter 7. The overall phase space model was also useful in understanding how likely it would be for a population that has been impacted by fish farming to fall into an ecological trap, be a population source or none. The model took into account two antagonistic effects (e.g. mortality and fecundity) that are difficult to measure in the field. For example, predation is difficult to measure in aquatic systems because it may take place in inaccessible depths, darkness and estimates of predation rates are not easy to measure (Gislason et al. 2010; Jørgensen et al. 2016). The model was useful in identifying data that is needed to be collected from the field.

The ecosystem-based model scenarios used in Chapter 8 were very useful in understanding what can happen in a system with the addition of fish and mussel farming. The model incorporated various trophic levels. Additionally, the model quantified the direct and indirect effects that one group can have on others. The model required a lot more data to be parametrised than the phase space models. Other limitations to the modelling approach are discussed in Chapter 8.

In both modelling approaches, single-species modelling and ecosystem-based modelling there was uncertainty based on the data available which is common using modelling techniques (Heymans et al. 2011). Model validation is often difficult as ecological interactions are complex which lead to low data accuracy (Codling and Dumbrell 2012). Although the models were not validated the overall results were consistent with some of the empirical data collected and the literature.

9.6.3 The use of pluralistic approach

As noted from this research there is a need of linking different research areas (e.g. anthropogenic resource subsidies, presence of physical structure) together in order to gain a more holistic view on ecological effects of aquaculture. The overall ecological impacts of aquaculture on marine fish communities are complex ranging from impacts at the individual, population and ecosystem levels.

Empirical research is necessary. The before/after control/impact (BACI) designs and the variety of BACI designs (e.g. multiple BACI (MBACI), paired BACIPS, beyond-BACI) are useful in evaluating any potential impacts of aquaculture on the wild fish. (Downes et al. 2002; Underwood 1992, 1997). Ideally, such designs would incorporate spatial and temporal variability associated with the natural environment (Underwood 1992, 1997). For this study, it was not possible to obtain data before the introduction of the fish farms in the lochs and therefore the designs were restricted to control/impact only which limits the conclusions of the study.

The use of BACI designs and the restrictions associated with such studies has resulted in concerns over increased rates of rejecting the null hypothesis when there is actually no impact (type I error) or accepting a null hypothesis when there is an impact (type II error) (Benedetti-Cecchi 2001; Murtaugh 2002). This has been noted to some extent in Chapters 4-6 when no differences were noted in the FCI of mackerel but when the data were pooled statistically significant differences were noted.

Stewart-Oaten et al. (1992) noted that it is more important to determine the size of an effect using statistical tools (e.g. using confidence intervals) rather than for significance hypothesis testing. Despite the limitations posed of only after impact studies conducted in Chapters 4-6, the data can be used to determine the size of the fish farming impact and improve future sampling designs. The data could also be used for simulating the fish farming impact on wild fish communities to determine the optimal sampling design (e.g. Benedetti-Cecchi 2001). Underwood (2009) also noted that having a combination of several small experiments is often preferred over larger experiments with more replication.

Future improved experimental designs should also take into account the statistical significance of an impact and the ecological relevance (Wilding and Hughes 2010). In general, the presence of an anthropogenic activity in the environment will have some level of localised impact with potential broad scale impacts (Wilding and Nickell 2007

and references therein). To detect any statistically significant impact of an anthropogenic activity such as fish farming on the environment depends on the number of observations, samples, temporal and spatial variability (Chapter 2; see also Wilding and Hughes 2010). The number of fish to be sampled near the sea cages and the number of replicates at the farm level depends on the purpose of the experiment and the logistics (Underwood 2009). This was noted in Chapters 4 and 5 where the logistics restricted either the number of fish to be analysed in the laboratory and/or the number of farms that could be visited.

Low sampling size (number of fish and number of sites) may not detect any statistically significant impacts and above a certain sampling size the cost and effort of collecting the data may result in wasting resources on detecting effects that are of such small magnitude as not to have practical consequences (see Underwood 2009; Wilding and Hughes 2010 and references therein). Thus, Wilding and Hughes (2010) pointed out there is need to assess the ecological importance of an impact rather than if there is an impact or not. The ecological importance is considered as the level at which an impact significantly affects the ecosystem. However, what the threshold of an ecologically relevant impact are has not been established yet (see Wilding and Hughes 2010 and references therein). The combination of different approaches could give a glimpse into the ecological levels at which aquaculture activities can cause a significant impact on the ecosystem.

When data is lacking or the logistics of running a fieldwork experiment are constraint modelling approached are very useful (e.g. Chapters 7 and 8). Collecting data at the population and ecosystem levels is costly and not possible in many cases. It is also worth noting that models can detect indirect effects that may not be as easy to detect by using fieldwork studies only. Models are also needed to understand the cumulative effect of aquaculture activities on the system and also taking into account presence of other anthropogenic activities. The combination of different approaches allows the collection of evidence from different perspectives which provides more robust conclusions.

9.7 Conclusions

The empirical and modelling studies described in this thesis aimed at understanding how aquaculture activities affect marine fish communities at the individual, population and ecosystem levels around coastal sea cages. By using a pluralistic approach evidence

collected at different levels allowed a more holistic view on the ecological impacts of aquaculture activities and how different disciplines relate to each other.

The two fish farms evaluated in this research provided the sampled fish a habitat rich in food resources which is reflected in an overall better biological condition. Mackerel, whiting and saithe sampled near the sea cages were found with waste pellets which was also reflected in their modified FA profiles. The overall effects of the two fish farms was more pronounced in young whiting and saithe than in mixed aged mackerel.

The phase space modelling approach indicated that the overall potential for fish farms to act at the extremes as either population sources or ecological traps are higher for juvenile whiting than for mackerel. Based on the empirical evidence and literature fish farms are more likely to be a population source for wild fishes. If that is the case, about 26% and 64% of the mackerel and whiting populations, respectively, visiting the cages would highly benefit in terms of growth. At a regional level, only about 0.20% and 0.47% of the mackerel and immature whiting would be affected.

Using an ecosystem modelling approach indicated that fish farming impacts the food web in a sea loch via nutrient loading. Mussel farming relies on the natural food resources and has the potential to affect the food web in a sea loch via competing with zooplankton for resources which can affect higher trophic levels. The presence of both activities can balance the impact on the food web of a sea loch when compared to the impact if these activities were present individually. Both activities have the potential do induce direct and indirect effects in the sea loch system.

The results of this work identified a number of gaps in data and thus could be used to improve future sampling designs. It is important to evaluate the cumulative effect of the presence of aquaculture activities in terms of nutrient loading and physical structure in the environment. Using a pluralistic approach to detect ecological effect of aquaculture activities is highly recommended.

Results of this PhD study could lead to more informed decisions in managing the coastal aquaculture activities. Establishing coastal fish farms as aquatic sanctuaries can be of an advantage to increase fish production and conserve species that are endangered provided that no commercial and recreational fishing is allowed nearby. It would be useful to have long term monitoring of the fish stocks around the cages and if there is any production at the regional level. Additionally, information on behavioural and migration patterns should be collected to understand further the impacts of aquaculture activities on fish stocks. From an aquaculture perspective, ecologically engineered fish farms in

addition to careful site selection in new aquaculture developments may improve nutrient loading into the ecosystem.

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APPENDIX A
MARINE ORGANISMS CAUGHT NEAR TWO FISH FARMS: CHAPTER 3

The following appendix includes information on underwater video recordings (A.1), macrobenthic sampling (A.2), environmental data collection (A.3), fish sampling (A.4), length at age for mackerel, saithe and whiting (A.5), and stomach content (A.6).

A.1 Underwater video recordings observations

The following observations were made during underwater video recordings in 2013 and 2014 at a halibut farm in Loch Melfort. Fish observed in video recordings included mackerel (~ 30-100 individuals) (Figure A.1), whiting (~ 40-50 individuals) (Figure A.2), juvenile clupeids (few hundred individuals in a school) (Figure A.3), goldsinny wrasse (*Ctenolabrus rupestris*), sandeel (Ammodytidae) (a shoal of ~ 15-30 individuals), poor cod (*Trisopterus minutus*), two-spotted goby (*Gobiusculus flavescens*).

The following was also noted on the camera: Atlantic mackerel (1 shoal of about 15-30 individuals) feeding on pellets lost from sea cages[16], mackerel feeding on juvenile clupeids[17] and juvenile clupeids feeding on plankton[18] and/or particulate organic matter.

The underwater videos at Kames bay farm showed some sheltered bedrocks colonised by anemone (*Protanthea simplex*) (Figure A.4), the common sea urchin (*Echinus esculentus*) (Figure A.5) and common sea star (*Asterias rubens*).

During underwater video recordings in July 2014 in both Loch Melfort and Loch Leven a number of moon jellyfish (*Aurelia aurita*) were noted with greater numbers in Loch Melfort[19] than in Loch Leven. Additionally, one or two lion's mane jellyfish (*Cyanea capillata*) (Figure A.6) were also observed in Loch Melfort.

Based on the underwater videos, the surface of the aquaculture cages at Kames Bay were colonized by sea squirts (Subphylum: Tunicata) and other organisms (Figure A.7).

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[16] Mackerel feeding on lost pellets: https://www.youtube.com/watch?v=IkVr5IDMnKQ [Accessed: 4 February 2018].
[17] Mackerel feeding on clupeids: https://www.youtube.com/watch?v=6q_5zBQGKoU [Accessed: 4 February 2018].
[18] Juvenile clupeids feeding on plankton: https://www.youtube.com/watch?v=9hxNnbxU8w4 [Accessed: 4 February 2018]
[19] Moon jellyfish in high numbers in Loch Melfort: https://www.youtube.com/watch?v=jr28dJC23z4 [Accessed: 4 February 2018].

A.2 Macrobenthic sampling

The main classes of macrobenthos found along a 1 km transect (see Chapter 3 for methodology) from the sea cages in Loch Melfort were Polychaeta (Families: Pectinariidae, Nereididae, Glyceridae, Phyllodocidea, Sabellidae, Cirratulidae, Nephtyidea, Pilargidae, Spionidae, Phyllocidae, Scalibregmatidae), Ophiuroidea (Family: Ophiurae), Asteroidea (Family: Asteriidae), Echinoidea (Family: Echinidae) (Table A.1). Lots of mussel shells were also noted under the sea cages. Common sea star and brittle stars can be found in Figures A.8 and A.9, respectively.

At the time of sampling (2013) the staff at Melfort farm caught common lobster (*Homarus gammarus*) (Figure A.10) and brown crab (*Cancer pagurus*) next to the sea cages (Figure A.11). Both the common lobster and the brown crab were caught using pots (Figure A.12). No data was collected on the common lobster and the brown crab as these were caught for non-scientific purposes.

A.3 Environmental data collection

During sampling in 2013 (September) at Melfort farm, the average dissolved oxygen concentration, temperature and salinity were 9.57 ± 0.16 mg/l, 13.7 ± 0.12 °C, and 34 ppt, respectively. The depth for all measurements was approximately 2 m from the surface. During sampling in 2014, the average temperature and salinity at Melfort farm were 13.2 ± 0.46°C and 33.5 ± 2.12 ppt at about 1 m depth, respectively. During the sampling period of 2014, the average temperature and salinity at 5 m depth at Leven farm were 13.88 ± 0.26°C and 29.08 ± 1.20 ppt, respectively.

A.4 Fish sampling

In this section, I describe the species caught during fieldwork of 2013 and 2014.

A.4.1 Fish sampling in 2013

The number and species of fish caught in 2013 are presented in Table A.2 and Figures A.13-A.18. Common skate (*Dipturus batis* L. (old name) split provisionally into *D. cf. flossada* and *D. cf. intermedia*; Lancaster et al. 2014) (Figure A.18) was caught using a different rod and line than the one used for mackerel and whiting. The skate was caught and released immediately after capture. The species caught in this study is most likely *D. cf. intermedia* (Lancaster et al. 2014). Two other adult female common skate

and a conger eel were caught and released by farm staff on different days. Mackerel was noted chasing after schools of clupeids during both fieldwork studies in 2013 and 2014.

A.4.2. Fish sampling in 2014

All fish collected in 2014 are described in Table A.3. Thornback ray (*Raja clavata*) (Figure A.19) and dogfish (*Scyliorhinus canicula*) (Figure A.20) caught at Loch Leven farm were released immediately after capture.

A.5 Length at age for mackerel, saithe and whiting

Length at age for each species (mackerel, saithe and whiting) was extracted from the ICES DATRAS online-database[20]. The average length at age for the years 2012-2014 is reported in Table A.4.

A.6 Stomach content

Fish pellets were found in stomachs of mackerel (Figure A.21, A.22), whiting (Figure A.23), saithe and dab (Figure A.24) caught near sea cages. Mackerel was often noted chasing after juvenile clupeids which was also evident in their stomach near and away from cages (Figure A.25). Juvenile shrimp and crabs (Figure A.26) were found in the stomachs of whiting caught near sea cages in Loch Melfort. Parasitic nematodes of the genus *Anisakis* were found in some of the mackerel caught near and away from cages (Figure A.27).

[20] ICES DATRAS: http://www.ices.dk/marine-data/data-portals/Pages/DATRAS.aspx [Accessed: 4 February 2018].

Table A.1 Macrobenthic sampling along a 1 km transect from a fish farm in Loch Melfort.

Sample	Distance from farm (m)	Depth (m)	No. of organisms	Phylum	Common name	Class	Family	Genus/Species
1	0	9.43±0.06	9	Annelida	polychaetes	Polychaeta	Pectinariidae	*Lagis koreni*
			2	Annelida	polychaetes	Polychaeta	Pectinariidae	unknown
			4	Annelida	polychaetes	Polychaeta	Nereididae	unknown
			1	Annelida	polychaetes	Polychaeta	Glyceridae	*Glycera alba*
			3	Annelida	polychaetes	Polychaeta	Phyllodocidea	unknown
			5	Annelida	polychaetes	Polychaeta	unknown	unknown
			4	Echinodermata	brittle stars	Ophiuroidea	unknown	unknown
			28	Echinodermata	brittle stars	Ophiuroidea	Ophiurae	*Ophiothrix fragilis*
			1	Echinodermata	brittle stars	Ophiuroidea	Ophiurae	*Ophiothrix nigra*
			1	Echinodermata	seastar	Asteroidea	Asteriidae	*Asterias rubens*
			1	Echinodermata	sea urchin	Echinoidea	Echinidae	unknown
2	20	23.5±0.71	3	Annelida	polychaetes	Polychaeta	unknown	unknown
			2	Annelida	polychaetes	Polychaeta	Pectinariidae	*Lagis koreni*
			2	Annelida	polychaetes	Polychaeta	Sabellidae	unknown
			57	Annelida	polychaetes	Polychaeta	Cirratulidae	*Chaetozone setosa*
			1	Echinodermata	brittle stars	Ophiuroidea	Ophiurae	unknown
3	60	31.0	3	Annelida	polychaetes	Polychaeta	Pectinariidae	*Lagis koreni*
			1	Annelida	polychaetes	Polychaeta	Nereididea	unknown
			2	Annelida	polychaetes	Polychaeta	Sabellaridae	unknown
			5	Annelida	polychaetes	Polychaeta	Nephtyidae	*Nephtys cirrosa*
			14	Annelida	polychaetes	Polychaeta	Cirratulidae	*Chaetozone setosa*
			1	Annelida	polychaetes	Polychaeta	unknown	unknown
			2	Echinodermata	Brittle stars	Ophiuroidea	unknown	unknown
4	535	29.5±6.36	1	Annelida	polychaetes	Polychaeta	Pectinariidae	*Lagis koreni*
			3	Annelida	polychaetes	Polychaeta	Pilargidae	*Ancistrosyllis groenlandica*
			1	Annelida	polychaetes	Polychaeta	Spionidae	unknown

			1	Annelida	polychaetes	Polychaeta	Phyllocidae	unknown
			1	Annelida	polychaetes	Polychaeta	Glyceridae	*Glycera rouxi*
5	952	41.0	12	Annelida	polychaetes	Polychaeta	Scalibregmatidae	*Scalibregma inflatum*
			11	Annelida	polychaetes	Polychaeta	Cirratulidae	*Chaetozone setosa*

Table A.2 Fish collected next to sea cages and at reference sites during fieldwork in September 2013.

Fish species	Common name	Melfort Farm	Reference Mackerel	Reference Saithe
Scomber scombrus	Atlantic mackerel	28	22	-
Merlangius merlangus	Whiting	32	4	-
Pollachius virens	Saithe	7	-	7
Gadus morhua	Cod	3	1	-
Limanda limanda	Dab	1	-	-
*Dipturus batis**	Common skate	1	-	-

*Released immediately after capture

Table A.3 Summary of fish collected during summer 2014.

Fish species	Common name	Melfort Farm	Leven Farm	Isle of Luing (Reference Mackerel)	Oban Bay (Reference Mackerel)	Mallaig (Reference Mackerel)	Firth of Clyde (Reference Whiting)	North Minch (Reference Whiting)
Scomber scombrus	Atlantic mackerel	110	17	69	67	45	-	-
Merlangius merlangus	Whiting	41	55	-	-	50	40	55
*Scyliorhinus canicula**	Dogfish	-	3	-	-	-	-	-
*Raja clavata**	Thornback ray	-	6	-	-	-	-	-
Gadus morhua	Cod	-	2	-	-	-	-	-
Pollachius virens	Saithe	8	3	-	1	-	-	-
Pollachius pollachius	Pollack	-	-	1	-	-	-	-
Eutrigla gurnardus	Grey gurnard	2	2	1	-	-	-	-
Limanda limanda	Dab	3	-	-	-	-	-	-
Trisopterus minutus	Poor cod	-	-	2	-	-	-	-
-	Goby	2	-	-	-	-	-	-

*Released immediately after capture

Table A.4. Length at age key for mackerel, saithe and whiting populations on the West coast of Scotland averaged for the years 2012-2014. Data is reported as mean and 95 % confidence intervals.

Age	Length (cm)		
	Mackerel	**Saithe**	**Whiting**
0	18.4 [18.0, 18.7]	.	16.5 [16.2, 16.9]
1	20.1 [19.8, 20.5]	32.2 [21.9, 42.5]	20.8 [20.5, 21.1]
2	25.3 [25.0, 25.5]	39.7 [38.1, 41.3]	28.1 [27.8, 28.4]
3	29.0 [28.7, 29.3]	43.8 [43.1, 44.5]	33.6 [33.3, 33.9]
4	31.4 [31.1, 31.8]	50.5 [49.7, 51.2]	37.5 [37.0, 37.9]
5	33.0 [32.6, 33.5]	56.3 [54.9, 57.7]	39.5 [38.8, 40.3]
6	34.3 [33.9, 34.8]	66.4 [62.8, 69.9]	41.5 [40.0, 43.0]
7	35.8 [35.0, 36.7]	78.2 [74.6, 81.8]	41.0 [39.1, 43.0]
8	36.4 [35.5, 37.2]	83.5 [81.0, 86.1]	37.7 [36.2, 39.1]
9	37.2 [36.0, 38.4]	91.3 [87.9, 94.7]	.
10	36.3 [34.6, 37.9]	96.3 [93.9, 98.6]	.
11	.	94.5 [92.2, 96.6]	54.0
12	39.3 [35.5, 43.1]	95.9 [93.1, 98.8]	.
13	.	98.4 [93.9, 103.0]	.
14	40.5	96.2 [92.1, 100.4]	.
15	41.0	96.6 [87.5, 105.7]	.
16	.	105.0 [41.5, 168.5]	.
17	.	100.0	.

Figure A.1 A school of mackerel (*Scomber scombrus*) noted on the underwater video recordings around the sea cages in Loch Melfort. Depth recorded: ~ 7 m from the water surface.

Figure A.2 Whiting (*Merlangius merlangus*) noted on the underwater video recordings around sea cages in Loch Melfort. Depth recorded: ~ 5 m from the water surface.

Figure A.3 School of clupeids noted on the underwater video recordings around the sea cages in Loch Melfort. Depth recorded: ~ 1 m from the water surface.

Figure A.4 Anemone (*Protanthea simplex*) near the sea cages at Loch Melfort noted on the underwater video recordings. Depth recorded: ~ 1.5 m from the water surface.

Figure A.5 Common sea urchin (*Echinus esculentus*) noted on the underwater video recordings near Loch Melfort. Depth recorded: ~ 1.5 m from the water surface.

Figure A.6 Jellyfish lion's mane jellyfish (*Cyanea capillata*) noted occasionally around sea cages in Loch Melfort.

Figure A.7 Sea squirts noted, using underwater video recordings, on the sea cages in Loch Melfort. Depth recorded: ~ 7 m from the water surface.

Figure A.8 Common sea star (*Asterias rubens*) caught under the sea cages in Loch Melfort.

Figure A.9 Brittle stars found near sea cages in Loch Melfort.

Figure A.10 Common lobster (*Homarus gammarus*) caught in pots in Loch Melfort.

Figure A.11 Brown crab (*Cancer pagurus*) caught in pots at Kames bay farm.

Figure A.12 Pots used to catch lobsters and brown crab near Kames bay farm.

Figure A.13 Atlantic mackerel (*Scomber scombrus*) caught near sea cages.

Figure A.14 Whiting (*Merlangius merlangus*) caught near sea cages.

Figure A.15 Saithe (*Pollachius virens*) caught near sea cages.

Figure A.16 Cod (*Gadus morhua*) caught near sea cages.

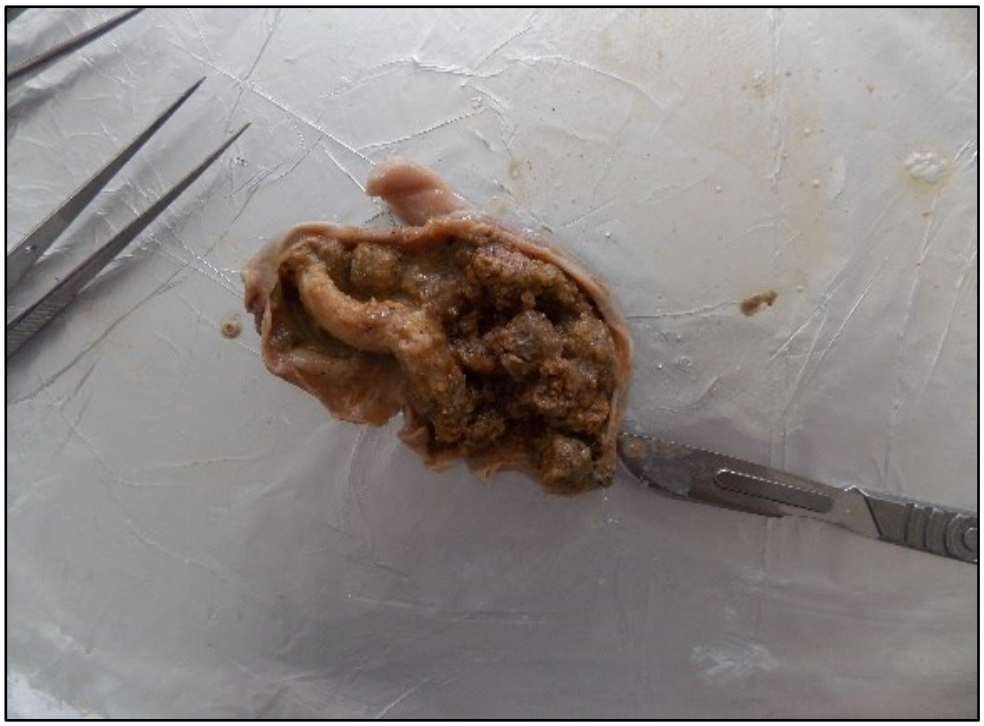
Figure A.17 Dab (*Limanda limanda*) caught near sea cages.

Figure A.18 Common skate (*Dipturus batis L. (old name); D. cf. intermedia*) caught near sea cages in Loch Melfort.

Figure A.19 Thornback ray (*Raja clavata*) caught and released near sea cages at Loch Leven during summer fieldwork of 2014.

Figure A.20 Dogfish (*Scyliorhinus canicula*) caught and released at the fish farm in Loch Leven during summer fieldwork 2014.

Figure A.21 Fish pellets found in mackerel (*Scomber scombrus*) stomachs collected at farm in Loch Melfort.



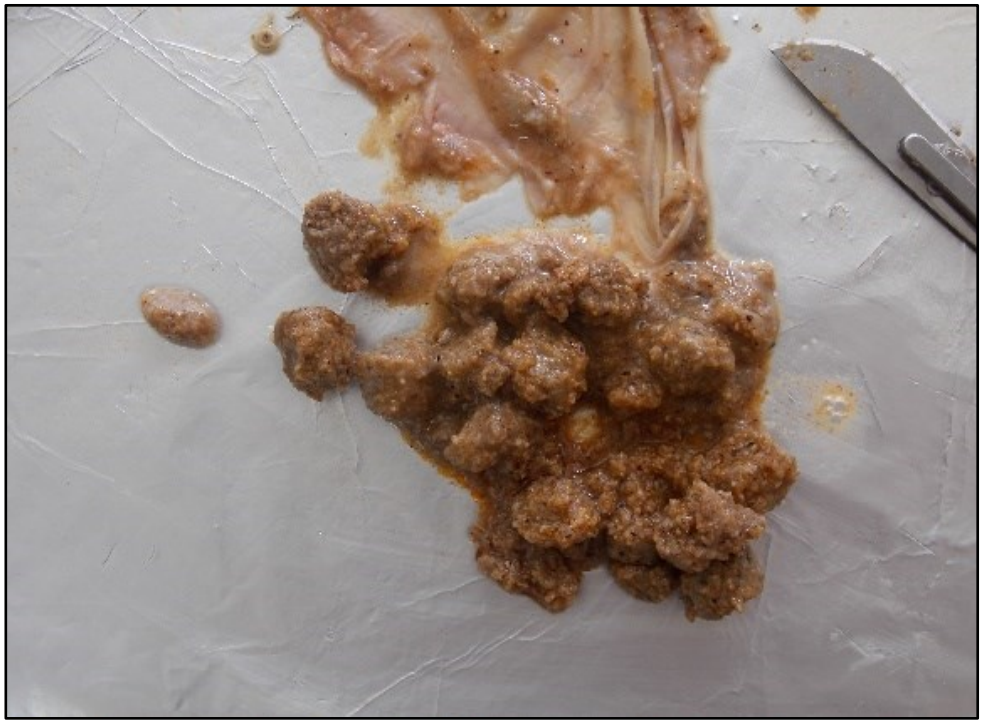


Figure A.22 Fish pellets found in mackerel (*Scomber scombrus*) stomachs collected at a fish farm in Loch Leven.

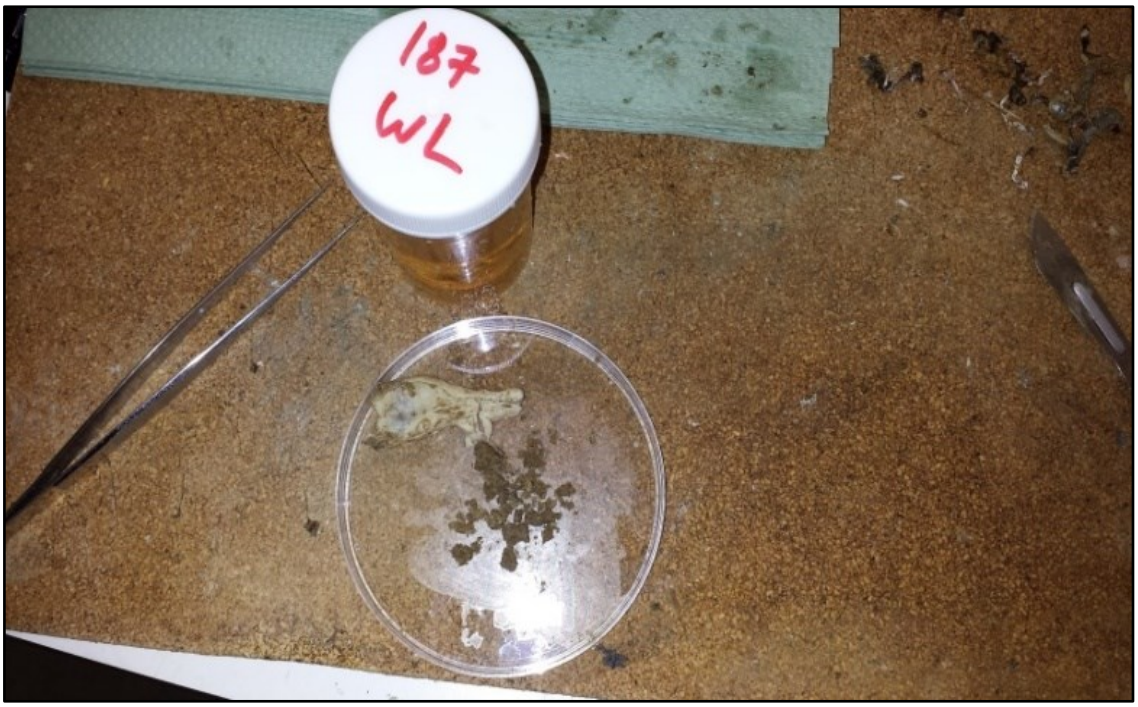


Figure A.23 Fish pellets found in juvenile whiting (*Merlangius merlangus*) stomach caught at sea cages of Leven Farm.

Figure A.24 Fish pellets in dab (*Limanda limanda*) stomachs caught at sea cages of Melfort Farm.

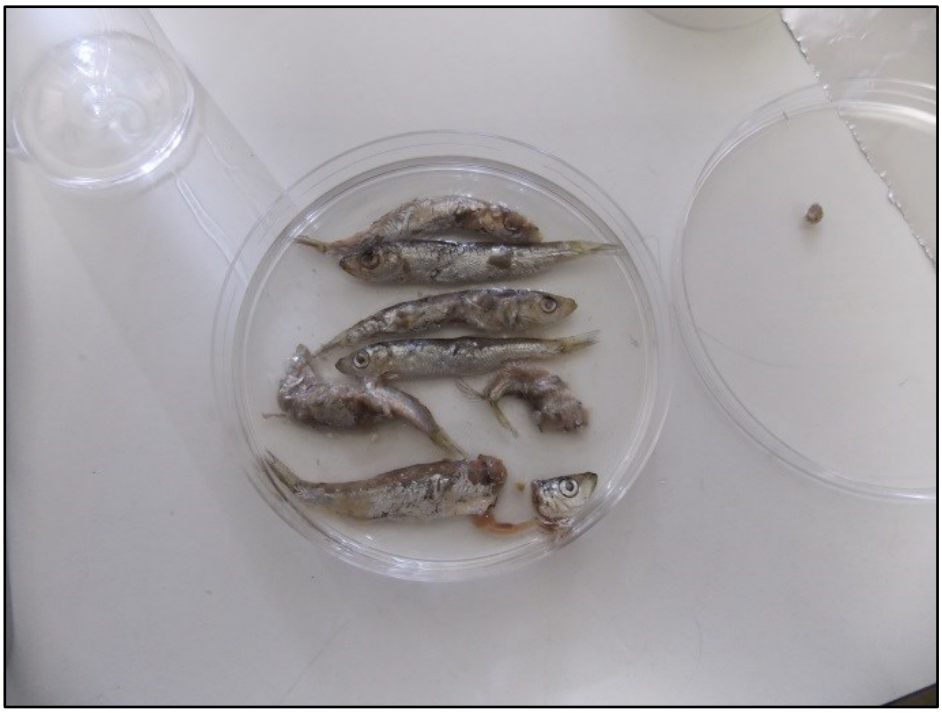


Figure A.25 Juvenile clupeids found in stomachs of mackerel near and away from cages.

Figure A.26 Juvenile crab found in stomach of whiting caught near sea cages in Loch Melfort.

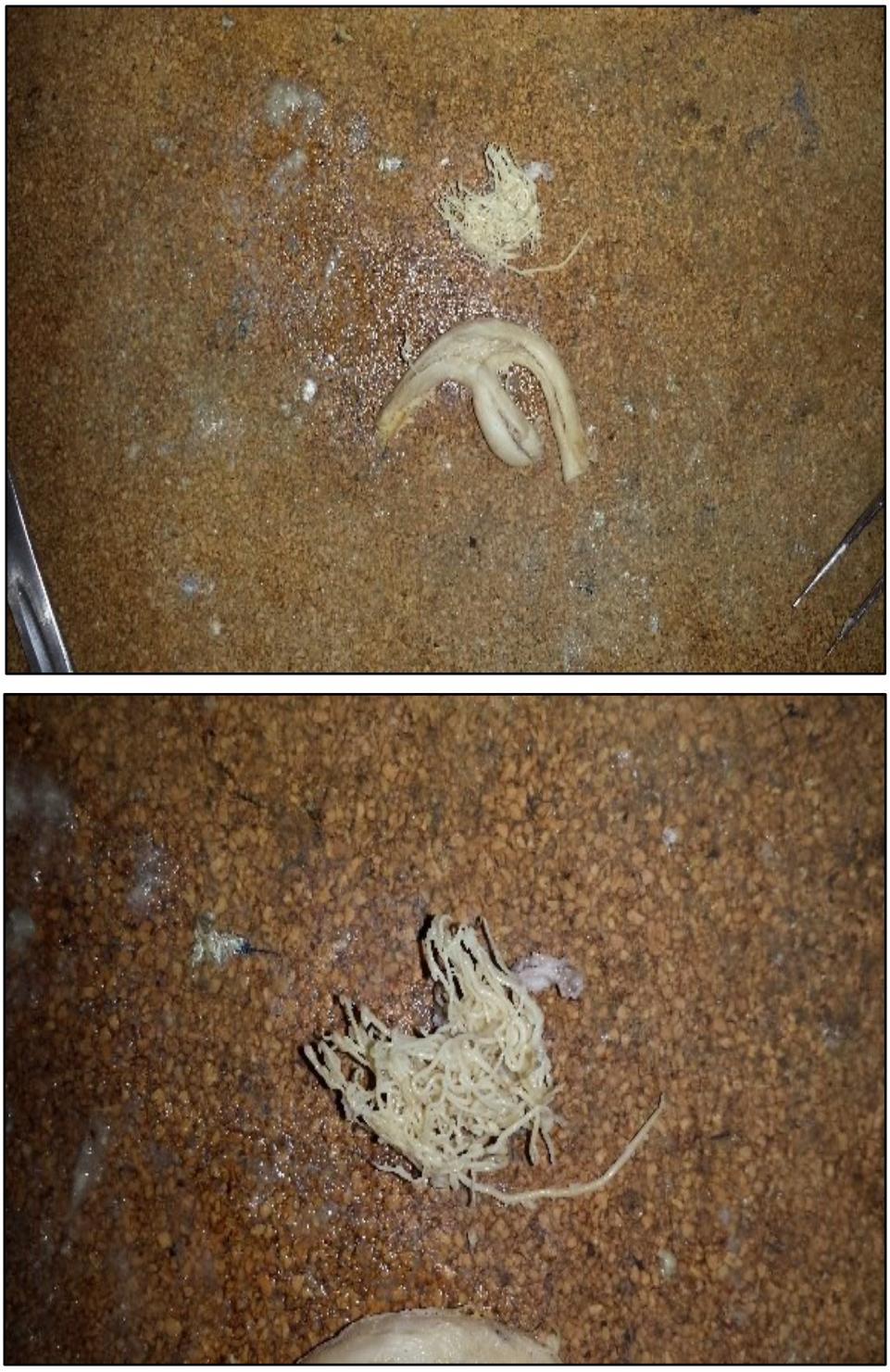


Figure A.27 Parasitic nematodes of the genus *Anisakis* found in some of the mackerel caught near and away from cages.

APPENDIX B

ADDITIONAL INFORMATION FOR CHAPTER 4

The following appendix provides information on residual plots for all the ANOVA tests run in Chapter 4. Additionally, the Appendix contains the full fatty acid (FA) profiles of muscle and liver tissues of mackerel and saithe caught near and away from a fish farm.

Table B.1 Total lipid (%) and fatty acid composition (%) of food pellets used to feed farmed fish and of muscle and liver tissues of mackerel caught next to and away from a fish farm in Loch Melfort. 95% confidence interval estimates of the sample means are presented.

	Diet	**Mackerel muscle**		**Mackerel liver**	
		Melfort Farm	**Reference Mackerel**	**Melfort Farm**	**Reference Mackerel**
No. of fish	1	11	10	11	10
Total Lipid	21.19 [21.16, 21.21]	9.72 [6.04, 13.4]	5.43 [3.65, 7.21]	12.14 [9.8, 14.47]	10.52 [9.64, 12.40]
Fatty Acids					
14:0	7.09 [6.77, 7.40]	2.75 [1.65, 3.86]	3.22 [2.68, 3.77]	0.60 [0.43, 0.77]	0.55 [0.43, 0.67]
15:0	0.48 [0.35, 0.61]	0.39 [0.29, 0.49]	0.65 [0.55, 0.75]	0.12 [0.06, 0.18]	0.10 [0.04, 0.16]
16:0	18.35 [16.83, 19.87]	17.83 [16.24, 19.42]	19.02 [18.36, 19.68]	18.36 [15.96, 20.75]	21.13 [19.86, 22.40]
18:0	3.66 [3.28, 4.04]	4.89 [4.06, 5.71]	5.19 [4.76, 5.63]	5.21 [4.24, 6.18]	6.14 [5.58, 6.70]
20:0	0.26 [0.13, 0.39]	0.23 [0.20, 0.25]	0.25 [0.24, 0.26]	0.19 [0.15, 0.22]	0.23 [0.22, 0.25]
22:0	0.18 [0.05, 0.31]	0.14 [0.10, 0.18]	0.13 [0.10. 0.15]	0.13 [0.11, 0.15]	0.18 [0.14, 0.22]
Total SFAs	30.02 [28.06, 31.99]	26.23 [24.66, 27.80]	28.47 [27.82, 29.12]	24.60 [21.40, 27.80]	28.33 [26.75, 29.91]
16:1n-9	0.16 [0.09, 0.41]	0.21[0.18, 0.24]	0.31 [0.29, 0.34]	0.26 [0.21, 0.31]	0.24 [0.19, 0.29]
16:1n-7	7.64 [6.30, 8.97]	4.00 [3.39, 4.62]	4.08 [3.64, 4.52]	3.11 [2.56, 3.65]	3.01 [2.53, 3.48]
18:1n-9	12.94 [12.11, 13.76]	21.43 [16.84, 26.01]	16.67 [13.26, 20.08]	37.69 [33.21, 42.16]	39.64 [35.42, 43.86]
18:1n-7	2.77 [2.45, 3.08]	4.35 [3.60, 5.09]	4.39 [3.96, 4.81]	7.47 [6.60, 8.33]	7.24 [6.69, 7.79]
20:1n-11	0.11 [0.00, 0.00]	0.39 [0.33, 0.46]	0.33 [0.24, 0.41]	0.43 [0.18, 0.68]	0.16 [0.08, 0.24]
20:1n-9	1.74 [1.68, 1.81]	3.84 [2.79, 4.89]	3.30 [2.74, 3.86]	3.85 [3.39, 4.30]	3.17 [2.54, 3.79]

20:1n-7	0.25 [0.25, 0.25]	0.42 [0.23, 0.61]	0.53 [0.45, 0.61]	0.31 [0.12, 0.50]	0.23 [0.14, 0.32]
22:1n-11	2.10 [1.72, 2.48]	4.25 [1.98, 6.51]	4.07 [2.87, 5.26]	1.72 [0.73, 2.72]	0.56 [0.34, 0.77]
22:1n-9	0.20 [-0.31, 0.71]	0.78 [0.45, 1.08]	0.63 [0.50, 0.76]	0.75 [0.64, 0.86]	0.72 [0.60, 0.85]
24:1n-9	0.53 [0.02, 1.04]	0.82 [0.75, 0.90]	0.88 [0.81, 0.95]	0.69 [0.62, 0.77]	0.73 [0.63, 0.84]
Total MUFAs	28.32 [25.78, 30.86]	40.48 [35.33, 45.62]	35.19 [32.05, 38.32]	56.27 [52.50, 60.04]	55.70 [51.66, 59.73]
18:2n-6	7.22 [7.03, 7.42]	3.22 [1.02, 5.43]	1.22 [1.00, 1.44]	2.27 [0.46, 4.08]	0.51 [0.19, 0.83]
18:3n-6	0.18 [0.18, 0.18]	0.14 [0.11, 0.18]	0.11 [0.09, 0.14]	0.15 [0.07, 0.24]	0.09 [0.04, 0.15]
20:2n-6	0.15 [0.08, 0.21]	0.28 [0.22, 0.33]	0.35 [0.29, 0.40]	0.33 [0.07, 0.60]	0.12 [0.02, 0.22]
20:3n-6	0.15 [-0.05, 0.34]	0.03 [[0.00, 0.06]	0.01 [-0.00, 0.03]	0.03 [-0.01, 0.06]	0.01 [-0.01, 0.04]
20:4n-6	0.97 [0.90, 1.03]	1.04 [0.82, 1.26]	1.01 [0.88, 1.15]	0.96 [0.66, 1.27]	0.69 [0.51, 0.87]
22:4n-6	0.04 [-0.47, 0.55]	0.13 [0.09, 0.16]	0.09 [0.06, 0.12]	0.15 [0.06, 0.24]	0.09 [0.02, 0.16]
22:5n-6	0.25 [0.25, 0.25]	0.29 [0.20, 0.38]	0.34 [0.28, 0.39]	0.13 [0.09, 0.18]	0.11 [0.06, 0.16]
Total n-6 PUFAs	8.95 [8.37, 9.52]	5.13 [2.94, 7.33]	3.13 [2.68, 3.59]	4.03 [1.61, 6.45]	1.63 [0.86, 2.40]
18:3n-3	1.09 [0.89, 1.28]	1.08 [0.53, 1.62]	0.95 [0.82, 1.07]	0.57 [0.14, 1.00]	0.21 [0.07, 0.35]
18:4n-3	2.11 [1.86, 2.36]	1.15 [0.78, 1.53]	1.76 [1.50, 2.01]	0.19 [0.10, 0.28]	0.13 [0.07, 0.21]
20:3n-3	0.00 [0.00, 0.00]	0.10 [0.06, 0.13]	0.17 [0.15, 0.19]	0.11 [0.01, 0.22]	0.05 [0.02, 0.12]
20:4n-3	0.63 [0.56, 0.67]	0.54 [0.44, 0.64]	0.68 [0.60, 0.76]	0.47 [0.23, 0.70]	0.39 [0.17, 0.61]
20:5n-3	13.56 [12.29, 14.83]	6.88 [6.08, 7.68]	8.31 [7.51, 9.11]	3.12 [2.38, 3.86]	2.62 [1.95, 3.28]
22:5n-3	1.70 [1.38, 2.01]	1.75 [1.58, 1.91]	1.71 [1.57, 1.86]	2.07 [1.03, 3.11]	1.46 [0.62, 2.31]
22:6n-3	9.58 [7.67, 11.49]	15.91 [11.16, 20.66]	18.93 [17.16, 20.69]	7.84 [6.33, 9.34]	8.74 [7.17, 10.31]
Total n-3 PUFAs	28.66 [24.58, 32.72]	27.65 [22.58, 32.23]	32.72 [29.90, 35.11]	14.37 [10.65, 18.08]	13.61 [10.26, 16.95]
16:2	1.00 [1, 1]	0.24 [0.17, 0.31]	0.22 [0.12, 0.32]	0.39 [0.25, 0.53]	0.41 [0.28, 0.54]
16:3	1.27 [1.27, 1.27]	0.23 [0.15, 0.30]	0.29 [0.16, 0.41]	0.33 [0.20, 0.45]	0.31 [0.20, 0.43]
16:4	1.79 [1.72, 1.84]	0.29 [0.20, 0.38]	0.20 [0.16, 0.24]	0.01 [0.00, 0.03]	0.01 [0.00, 0.03]
Total	4.06 [3.93, 4.19]	0.75 [0.58, 0.93]	0.71 [0.57, 0.85]	0.73 [0.46, 1.00]	0.73 [0.48, 0.99]
Total PUFAs	41.66 [37.14, 46.17]	33.29 [28.40, 38.19]	36.34 [33.39, 39.30]	19.12 [13.11, 25.14]	15.97 [11.66, 20.28]
n-3/n-6	3.20 [2.95, 3.45]	7.54 [4.78, 10.30]	10.63 [9.52, 11.75]	5.56 [3.51, 7.61]	9.68 [7.51, 11.85]

Table B.2 Total lipid (%) and fatty acid composition (%) of food pellets used to feed farmed fish and of muscle and liver tissues of saithe caught next to and away from a fish farm in Loch Melfort. 95% confidence interval estimates of the sample means are presented.

	Diet	**Saithe muscle**		**Saithe liver**	
		Melfort Farm	**Reference Saithe**	**Melfort Farm**	**Reference Saithe**
No. of fish	1	7	7	7	7
Total Lipid	21.19 [21.16, 21.21]	0.98 [0.94, 1.07]	1.11 [1.05, 1.19]	47.17 [42.28, 52.05]	46.47 [40.21, 54.35]
Fatty Acids					
14:0	7.09 [6.77, 7.40]	1.28 [1.01, 1.54]	0.94 [0.84, 1.03]	2.50 [1.99, 3.00]	1.98 [1.76, 2.20]
15:0	0.48 [0.35, 0.61]	0.36 [0.30, 0.42]	0.34 [0.31, 0.36]	0.45 [0.33, 0.57]	0.47 [0.43, 0.51]
16:0	18.35 [16.83, 19.87]	17.72 [17.29, 18.16]	17.02 [16.62, 17.43]	14.68 [13.93, 15.44]	15.44 [14.61, 16.27]
18:0	3.66 [3.28, 4.04]	5.65 [5.37, 5.92]	6.39 [6.13, 6.64]	6.51 [5.74, 7.28]	6.27 [5.53, 7.00]
20:0	0.26 [0.13, 0.39]	0.05 [0.02, 0.09]	0.03 [-0.01, 0.06]	0.17 [0.12, 0.22]	0.12 [0.10, 0.15]
22:0	0.18 [0.05, 0.31]	0.04 [0.01, 0.07]	0.03 [-0.01, 0.07]	0.11 [0.06, 0.16]	0.03 [-0.01, 0.07]
Total SFAs	30.02 [28.06, 31.99]	25.10 [24.39, 25.82]	24.74 [24.31, 25.17]	24.42 [23.27, 25.57]	24.31 [23.66, 24.96]
16:1n-9	0.16 [0.09, 0.41]	0.25 [0.23, 0.28]	0.30 [0.26, 0.34]	0.31 [0.17, 0.45]	0.41 [0.38, 0.43]
16:1n-7	7.64 [6.30, 8.97]	1.83 [1.44, 2.23]	1.61 [1.46, 1.77]	4.51 [3.23, 5.78]	3.83 [3.27, 4.39]
18:1n-9	12.94 [12.11, 13.76]	11.09 [9.94, 12.24]	11.24 [10.78, 11.70]	22.03[19.63, 24.44]	19.68 [18.04, 21.33]
18:1n-7	2.77 [2.45, 3.08]	2.74 [2.48, 3.00]	2.88 [2.82, 2.94]	4.23 [3.78, 4.68]	4.46 [4.38, 4.55]
20:1n-11	0.11 [0.00, 0.00]	0.22 [0.15, 0.30]	0.37 [0.28, 0.45]	0.57 [0.10, 1.04]	0.95 [0.59, 1.31]
20:1n-9	1.74 [1.68, 1.81]	1.41 [1.21, 1.62]	1.28 [1.15, 1.42]	2.93 [2.38, 3.48]	2.58 [2.06, 3.10]
20:1n-7	0.25 [0.25, 0.25]	0.17 [0.07, 0.26]	0.25 [0.21, 0.29]	0.64 [0.05, 1.23]	0.75 [0.39, 1.11]
22:1n-11	2.10 [1.72, 2.48]	0.71 [0.53, 0.88]	0.71 [0.54, 0.88]	1.95 [1.35, 2.54]	1.61 [0.76, 2.45]
22:1n-9	0.20 [-0.31, 0.71]	0.15 [0.13, 0.17]	0.19 [0.16, 0.22]	0.28 [0.20, 0.37]	0.18 [0.14, 0.21]
24:1n-9	0.53 [0.02, 1.04]	0.50 [0.43, 0.57]	0.59 [0.53, 0.65]	0.39 [0.31, 0.47]	0.44 [0.32, 0.56]
Total MUFAs	28.32 [25.78, 30.86]	19.08 [17.35, 20.80]	19.42 [18.86, 19.98]	37.84 [35.00, 40.68]	34.89 [33.97, 35.81]
18:2n-6	7.22 [7.03, 7.42]	2.98 [2.09, 3.86]	1.91 [1.53, 2.29]	6.02 [4.47, 7.57]	3.50 [1.86, 5.14]
18:3n-6	0.18 [0.18, 0.18]	0.17 [0.14, 0.21]	0.14 [0.12, 0.17]	0.13 [0.09, 0.17]	0.11 [0.08, 0.14]
20:2n-6	0.15 [0.08, 0.21]	0.66 [0.31, 1.01]	0.78 [0.54, 1.02]	1.05 [-0.05, 2.15]	1.11 [0.57, 1.66]

20:3n-6	0.15 [-0.05, 0.34]	0.14 [0.22, 0.25]	0.11 [0.02, 0.19]	0.16 [0.02, 0.31]	0.12 [0.02, 0.22]
20:4n-6	0.97 [0.90, 1.03]	2.55 [2.12, 2.98]	2.69 [2.42, 2.96]	1.43 [1.07, 1.79]	1.33 [1.16, 1.49]
22:4n-6	0.04 [-0.47, 0.55]	0.20 [0.06, 0.34]	0.27 [0.22, 0.32]	0.17 [-0.00, 0.34]	0.27 [0.22, 0.32]
22:5n-6	0.25 [0.25, 0.25]	0.53 [0.48, 0.59]	0.44 [0.35, 0.52]	0.22 [0.18, 0.27]	0.24 [0.19, 0.28]
Total n-6 PUFAs	8.95 [8.37, 9.52]	7.23 [6.33, 8.14]	6.33 [5.52, 7.14]	9.17 [7.32, 11.03]	6.67 [4.54, 8.81]
18:3n-3	1.09 [0.89, 1.28]	0.74 [0.53, 0.95]	0.69 [0.62, 0.76]	1.46 [1.02, 1.90]	1.46 [1.18, 1.73]
18:4n-3	2.11 [1.86, 2.36]	0.52 [0.38, 0.65]	0.60 [0.45, 0.74]	1.44 [1.15, 1.73]	1.77 [1.34, 2.20]
20:3n-3	0.00 [0.00, 0.00]	0.19 [0.11, 0.28]	0.27 [0.20, 0.34]	0.30 [0.07, 0.52]	0.42 [0.25, 0.60]
20:4n-3	0.63 [0.56, 0.67]	0.49 [0.44, 0.55]	0.50 [0.46, 0.53]	0.57 [0.50, 0.64]	0.65 [0.56, 0.73]
20:5n-3	13.56 [12.29, 14.83]	15.05 [14.35, 15.75]	14.31 [13.38, 15.23]	12.31 [10.06, 14.56]	12.69 [11.69, 13.69]
22:5n-3	1.70 [1.38, 2.01]	1.80 [1.61, 1.98]	2.36 [1.96, 2.76]	1.23 [0.97, 1.50]	2.04 [1.60, 2.48]
22:6n-3	9.58 [7.67, 11.49]	29.27 [27.15, 31.39]	30.17 [28.37, 31.97]	10.24 [8.04, 12.45]	14.44 [11.67, 17.21]
Total n-3 PUFAs	28.66 [24.58, 32.72]	48.06 [46.37, 49.75]	48.89 [47.95, 49.83]	27.55 [23.39, 31.70]	33.46 [30.88, 36.05]
16:2	1.00 [1, 1]	0.15 [0.10, 0.20]	0.22 [0.16, 0.28]	0.33 [0.17, 0.49]	0.25 [0.21, 0.28]
16:3	1.27 [1.27, 1.27]	0.19 [0.15, 0.22]	0.21 [0.19, 0.23]	0.35 [0.12, 0.58]	0.21 [0.17, 0.25]
16:4	1.79 [1.72, 1.84]	0.18 [0.16, 0.21]	0.19 [0.17, 0.21]	0.34 [0.06, 0.62]	0.20 [0.17, 0.23]
Total 16	4.06 [3.93, 4.19]	0.52 [0.47, 0.57]	0.62 [0.56, 0.68]	1.03 [0.37, 1.68]	0.66 [0.55, 0.77]
Total PUFAs	41.66 [37.14, 46.17]	55.81 [54.01, 57.63]	55.85 [55.11, 56.57]	37.75 [34.03, 41.46]	40.80 [39.42, 42.18]
n-3/n-6	3.20 [2.95, 3.45]	6.75 [5.88, 7.63]	7.88 [6.59, 9.18]	3.17 [2.31, 4.03]	5.90 [3.08, 8.72]

Figure B.1 Residual plots for the analysis of variance (ANOVA) models for length, mass, FCI, and HSI for mackerel sampled near and away from a fish farm in Loch Melfort.

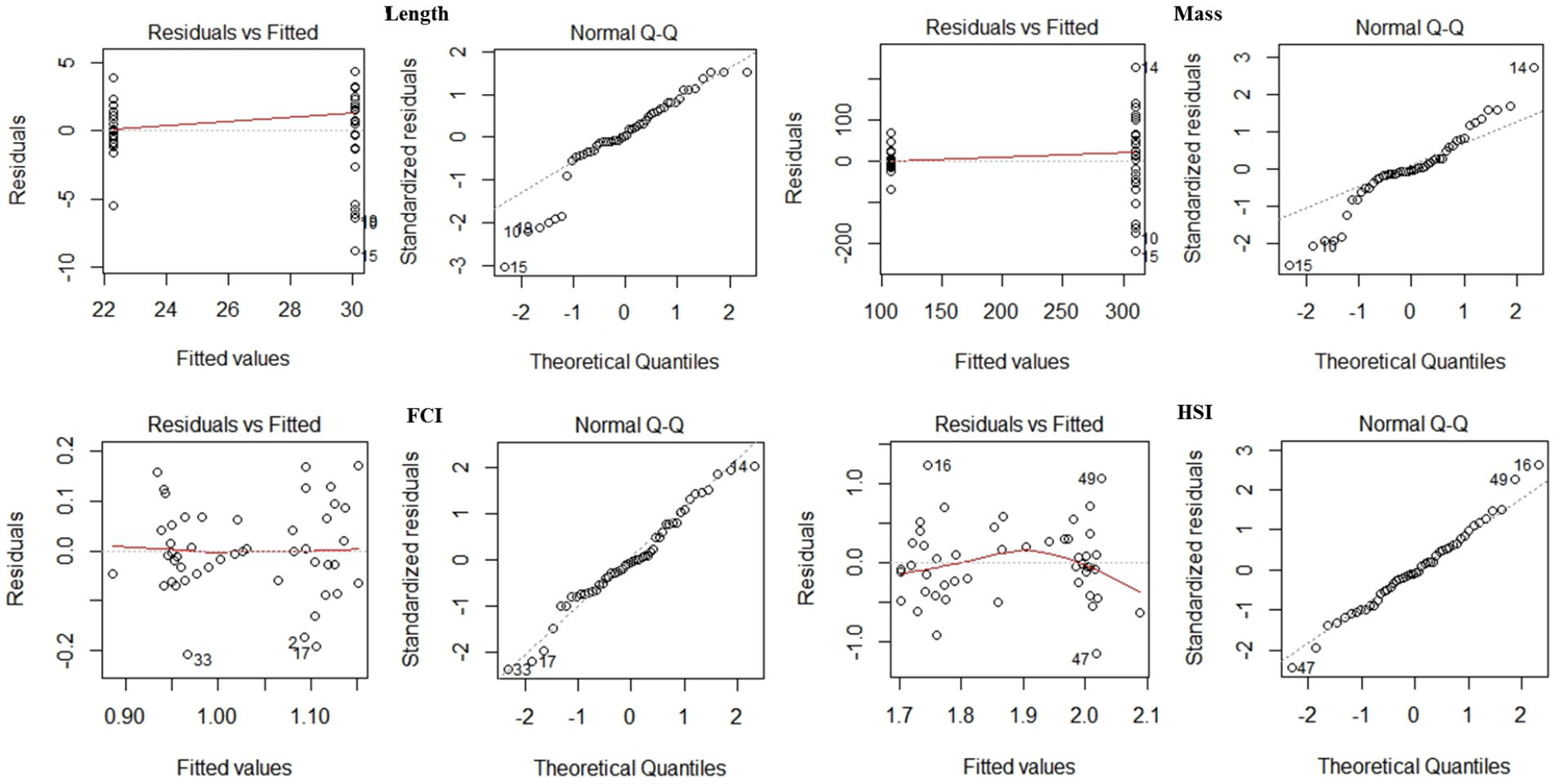


Figure B.2 Residual plots for the analysis of variance (ANOVA) models for length, mass, FCI, HSI for saithe sampled near and away from a fish farm in Loch Melfort.

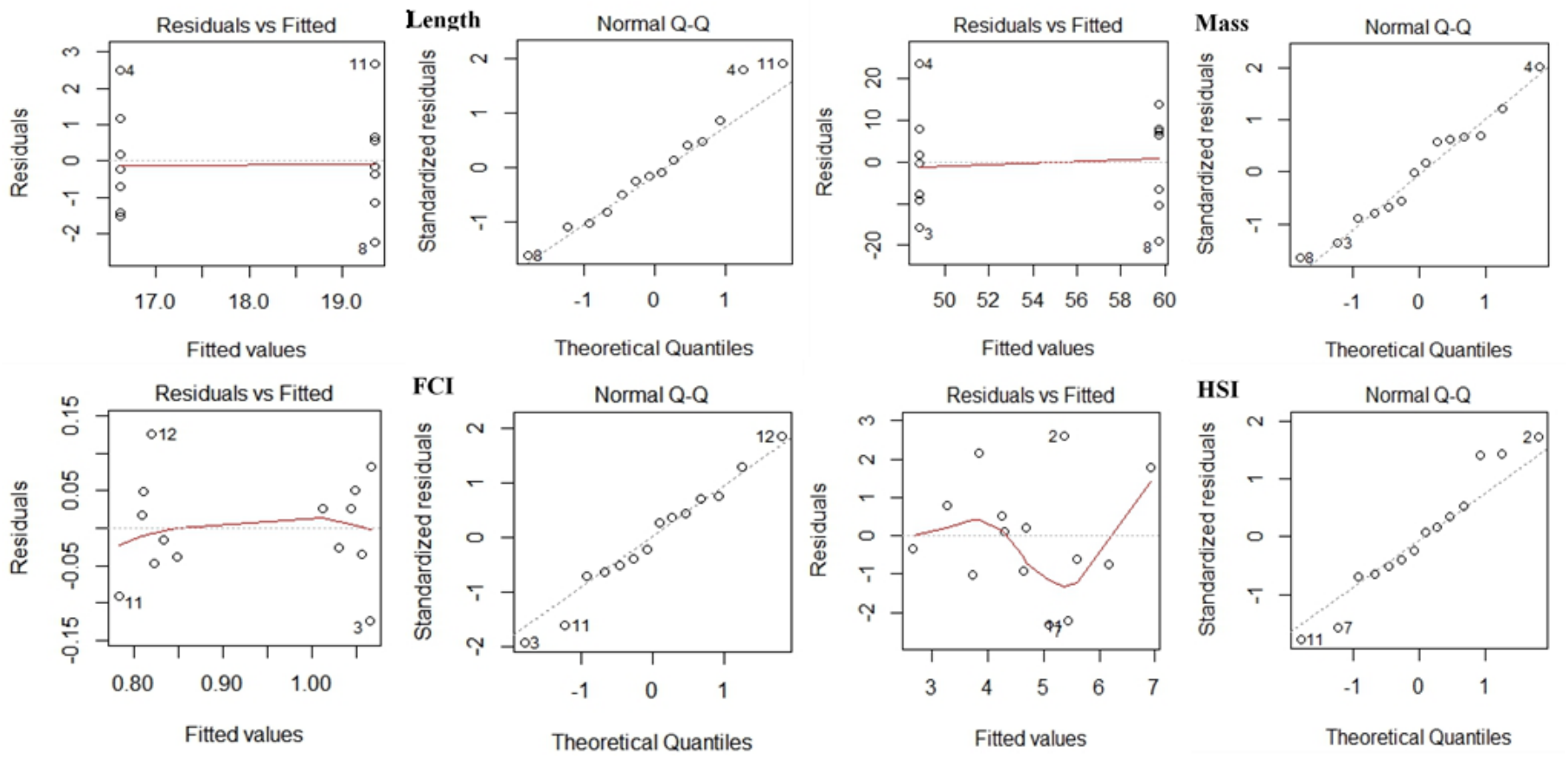
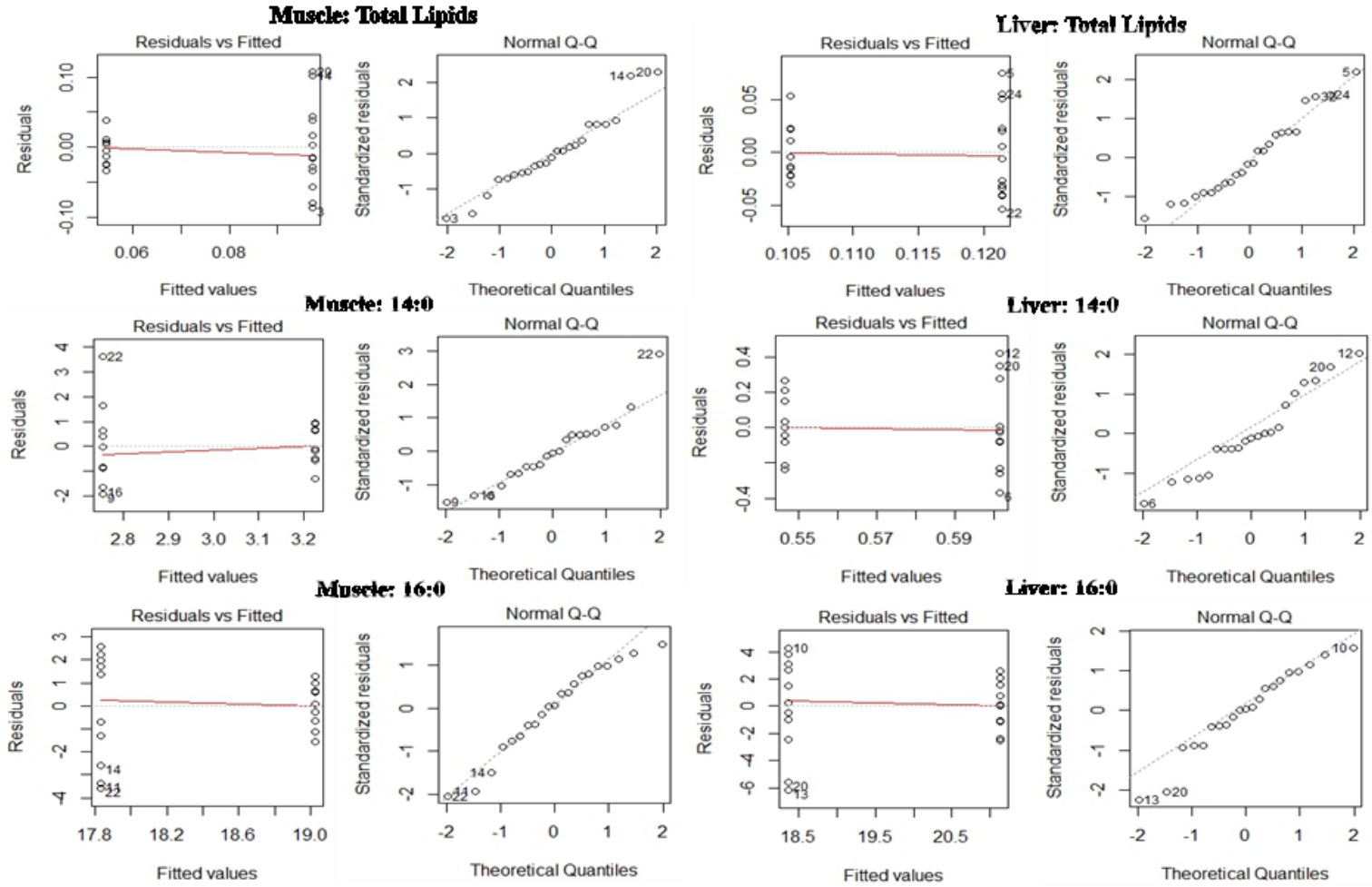
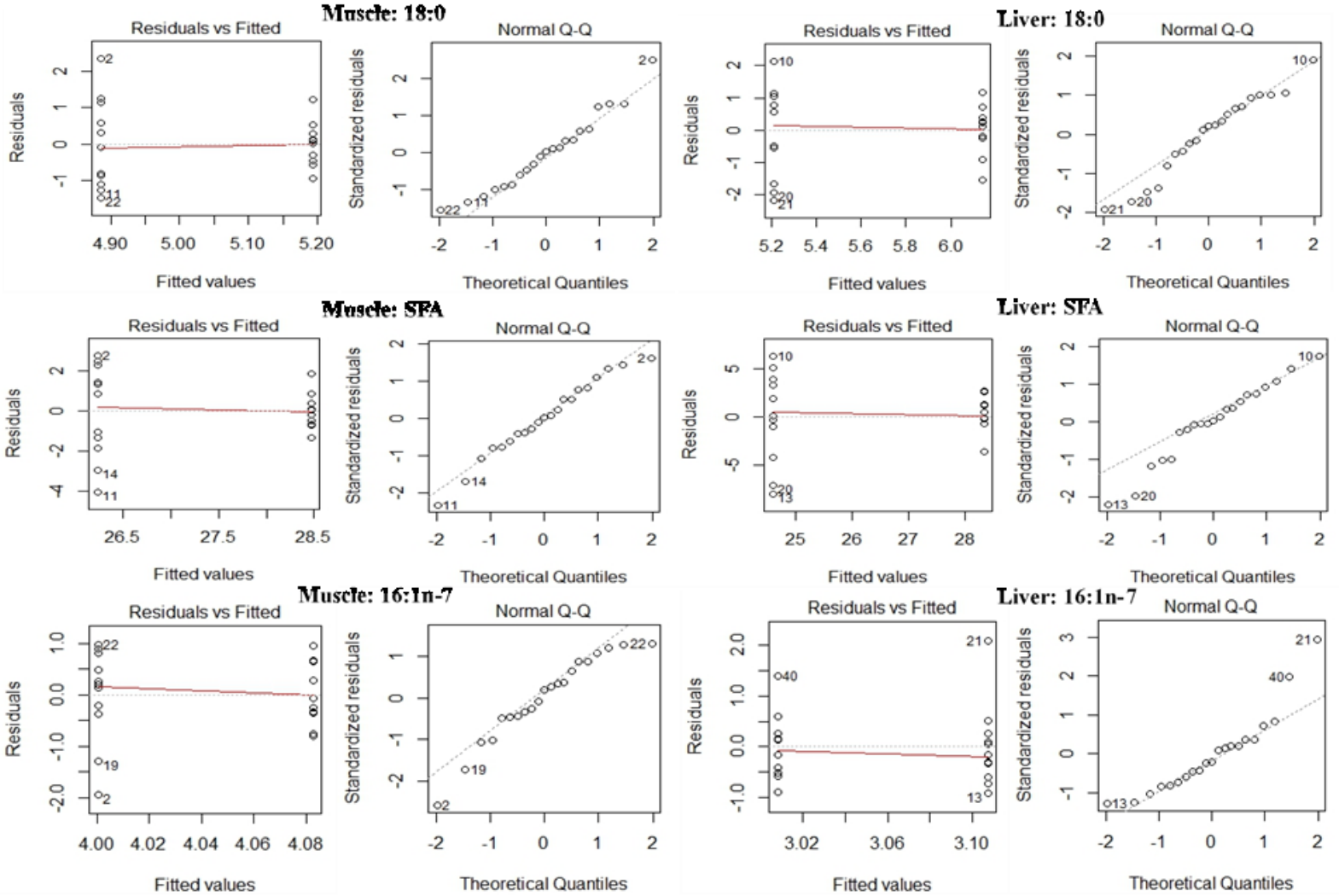


Figure B.3 Residual plots for the analysis of variance (ANOVA) models for total lipids and fatty acids in mackerel muscle and liver tissues sampled near and away from a farm.

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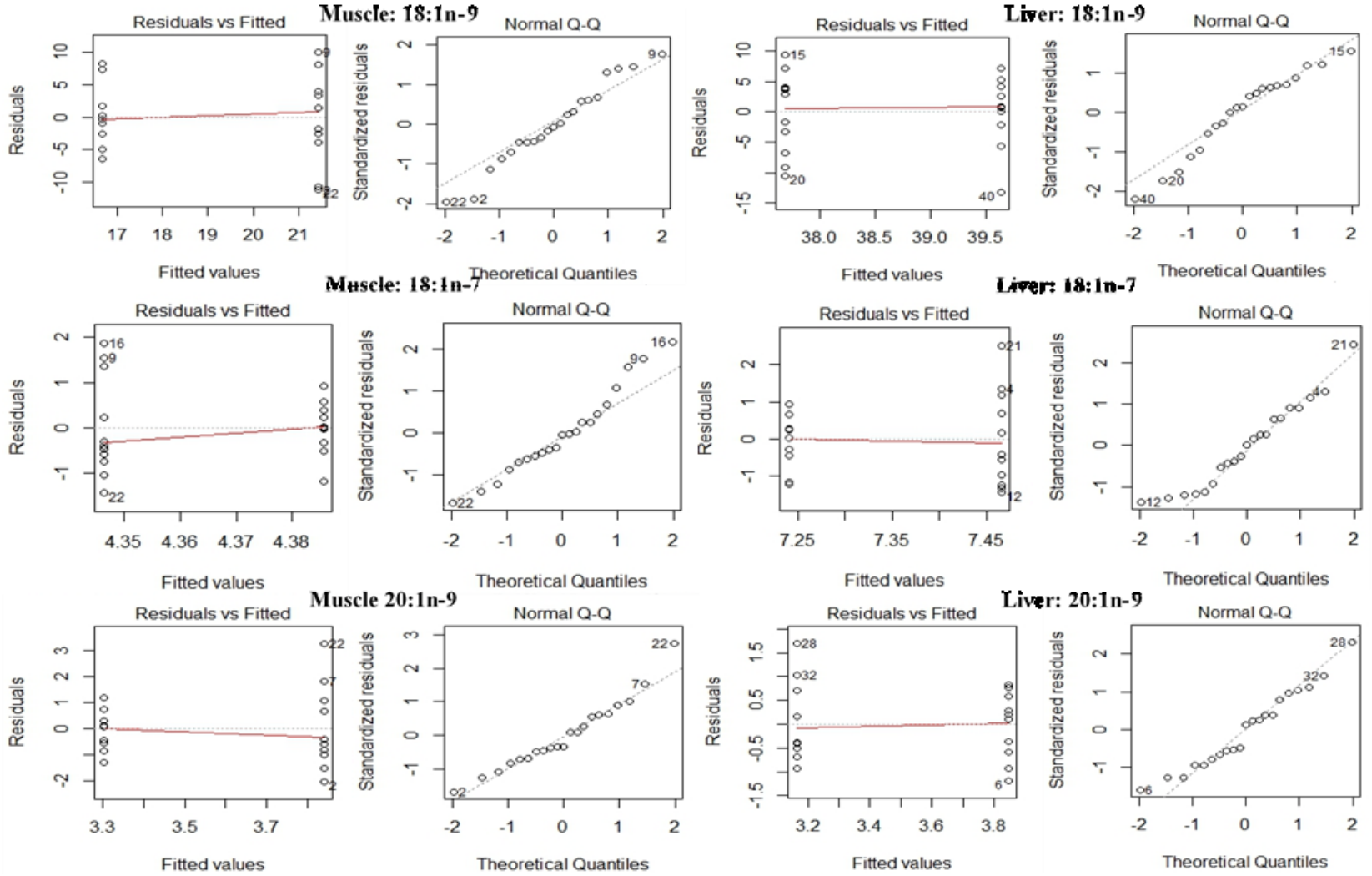
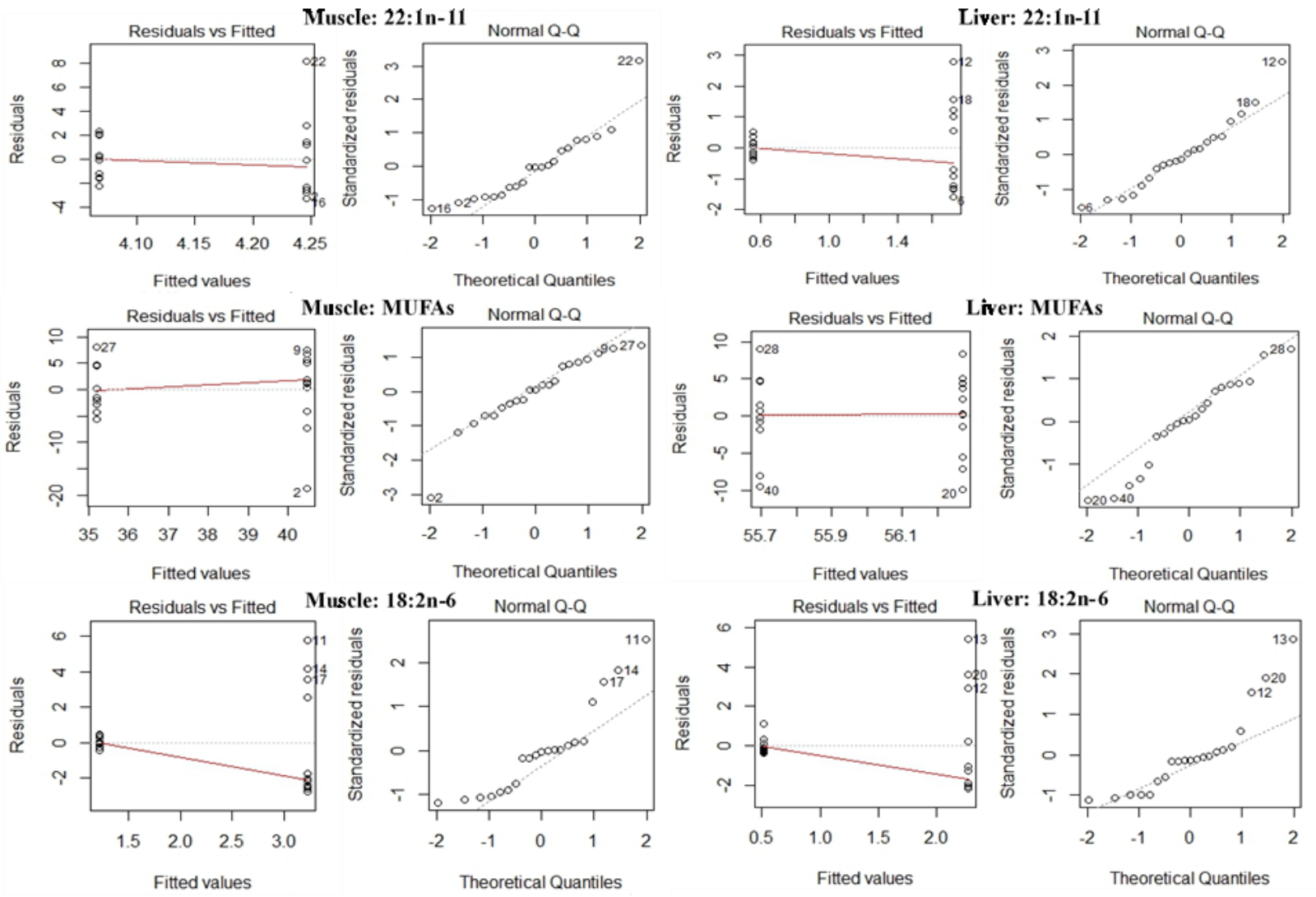
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:0
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: SFA
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: SFA
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 16:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 16:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles


Muscle: 18:1n-9
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:1n-9
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 18:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle 20:1n-9
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 20:1n-9
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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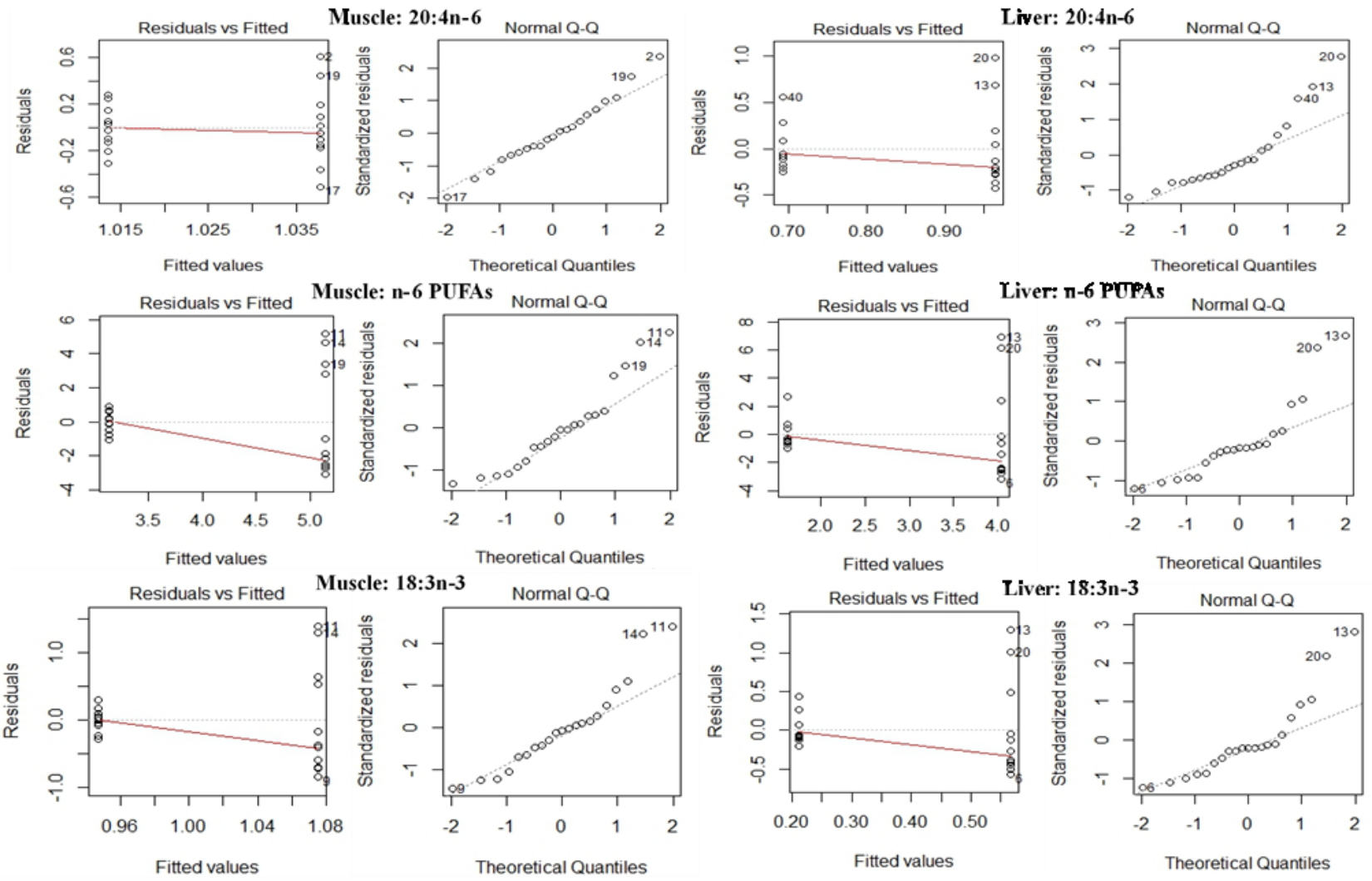
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:1n-11
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: MUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: MUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 18:2n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:2n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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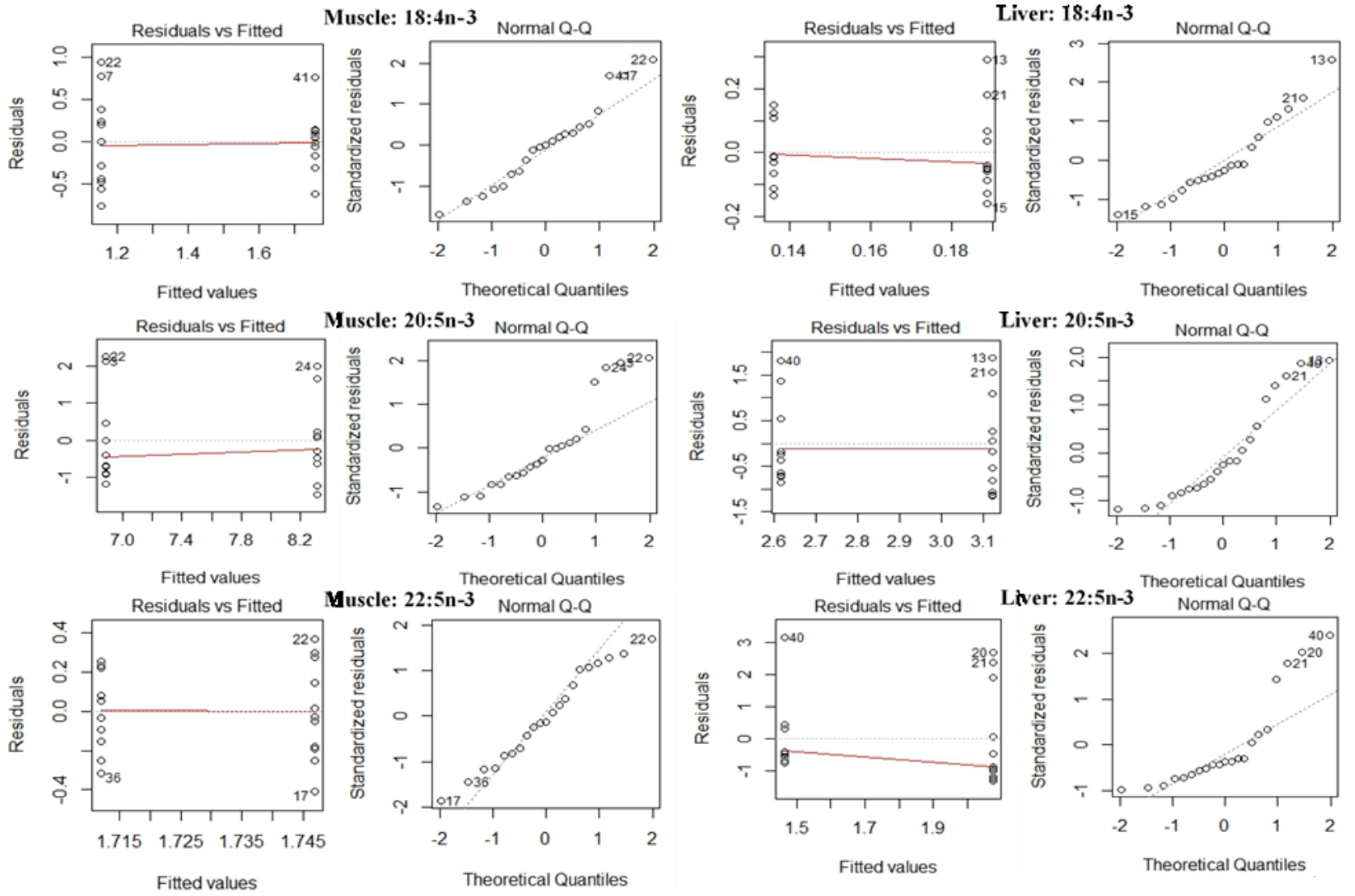
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 20:4n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: n-6 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: n-6 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 18:3n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:3n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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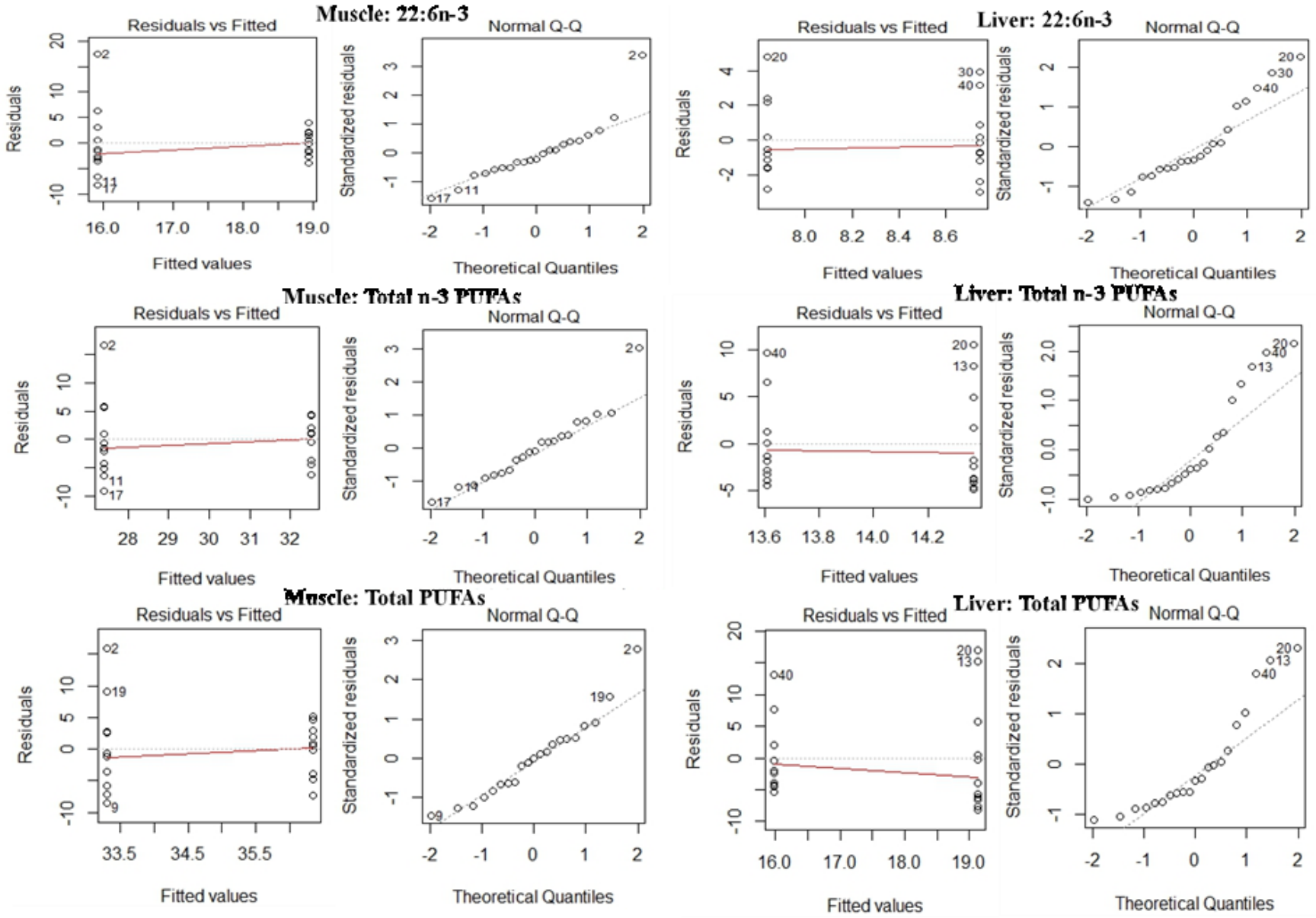
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:4n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 20:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 20:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 22:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles


Muscle: 22:6n-3
Residuals vs Fitted
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Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:6n-3
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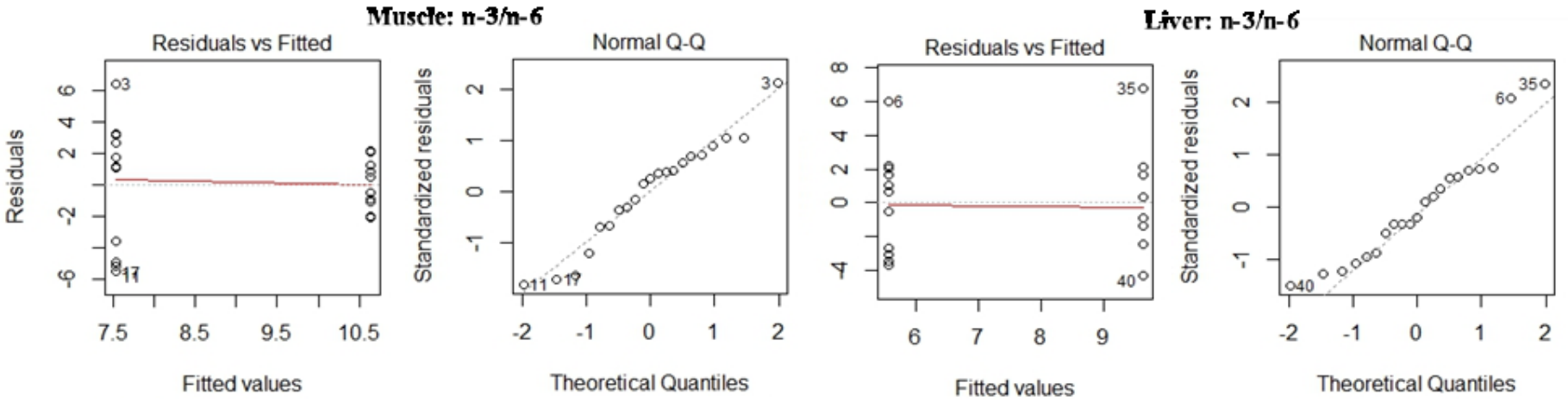
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: Total n-3 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: Total n-3 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: Total PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: Total PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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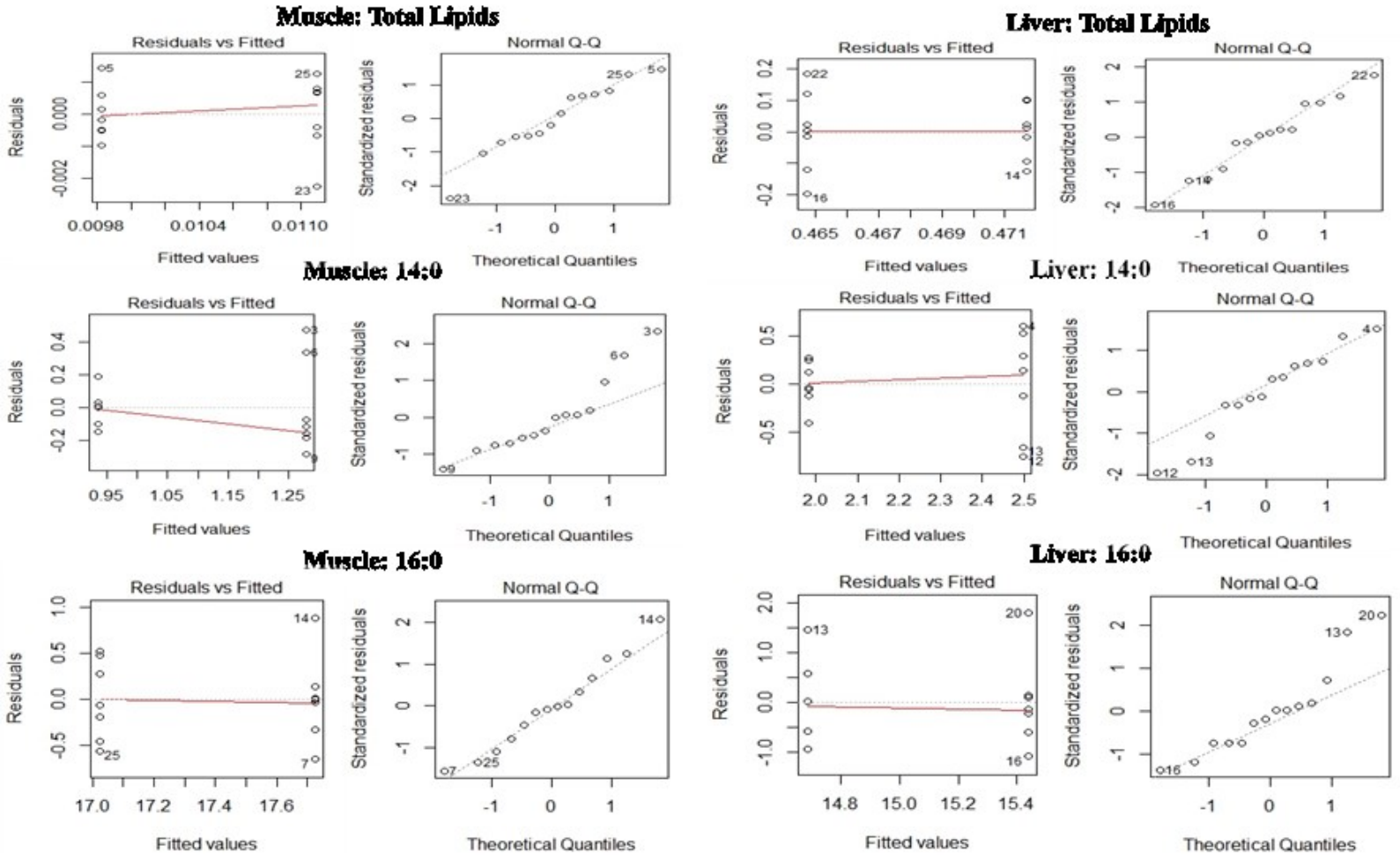
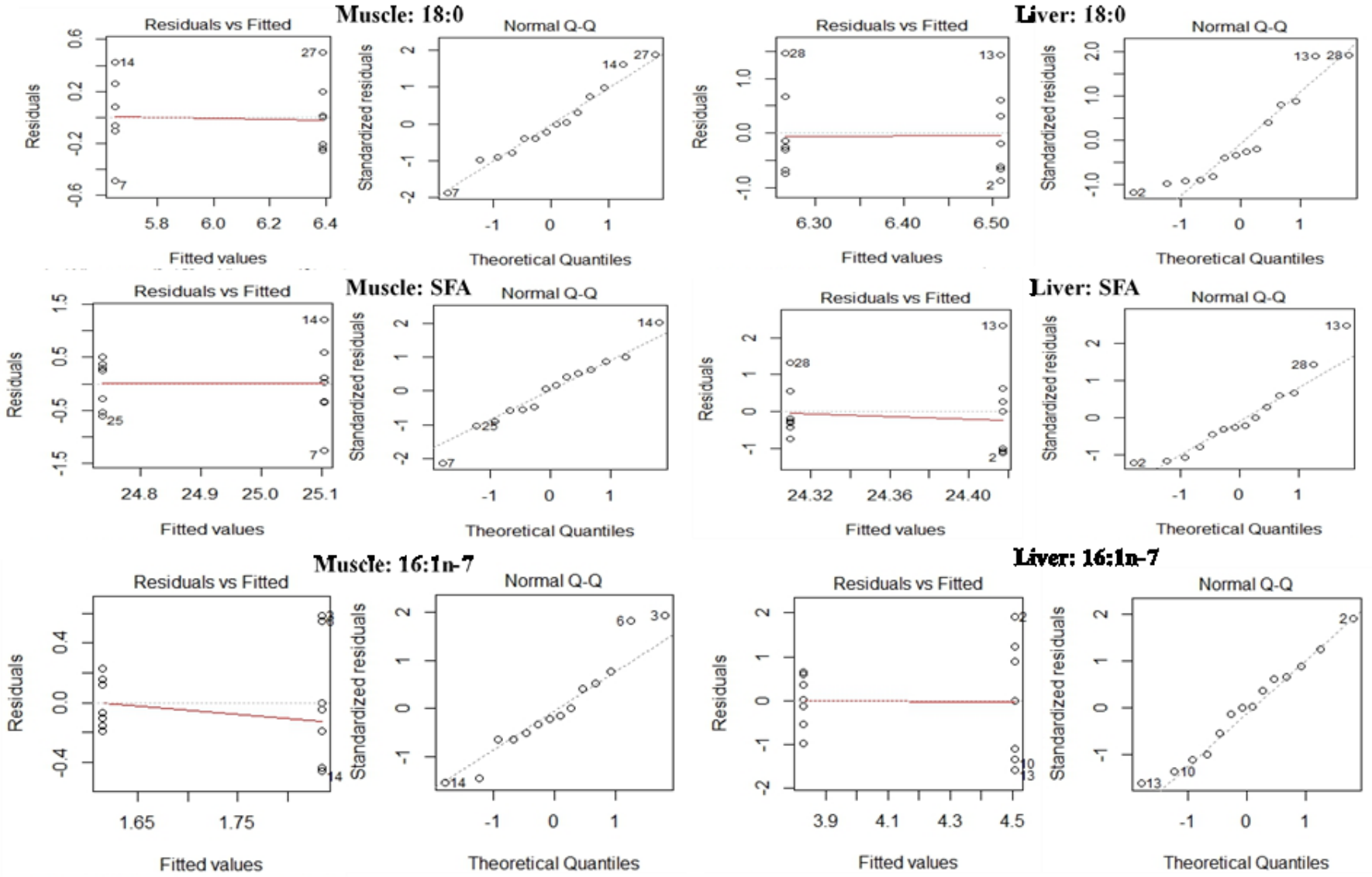
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: n-3/n-6
Residuals vs Fitted
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles

Figure B.4 Residual plots for the analysis of variance (ANOVA) models for total lipids and fatty acids in saithe muscle and liver tissues sampled near and away from a farm.




Muscle: 18:0
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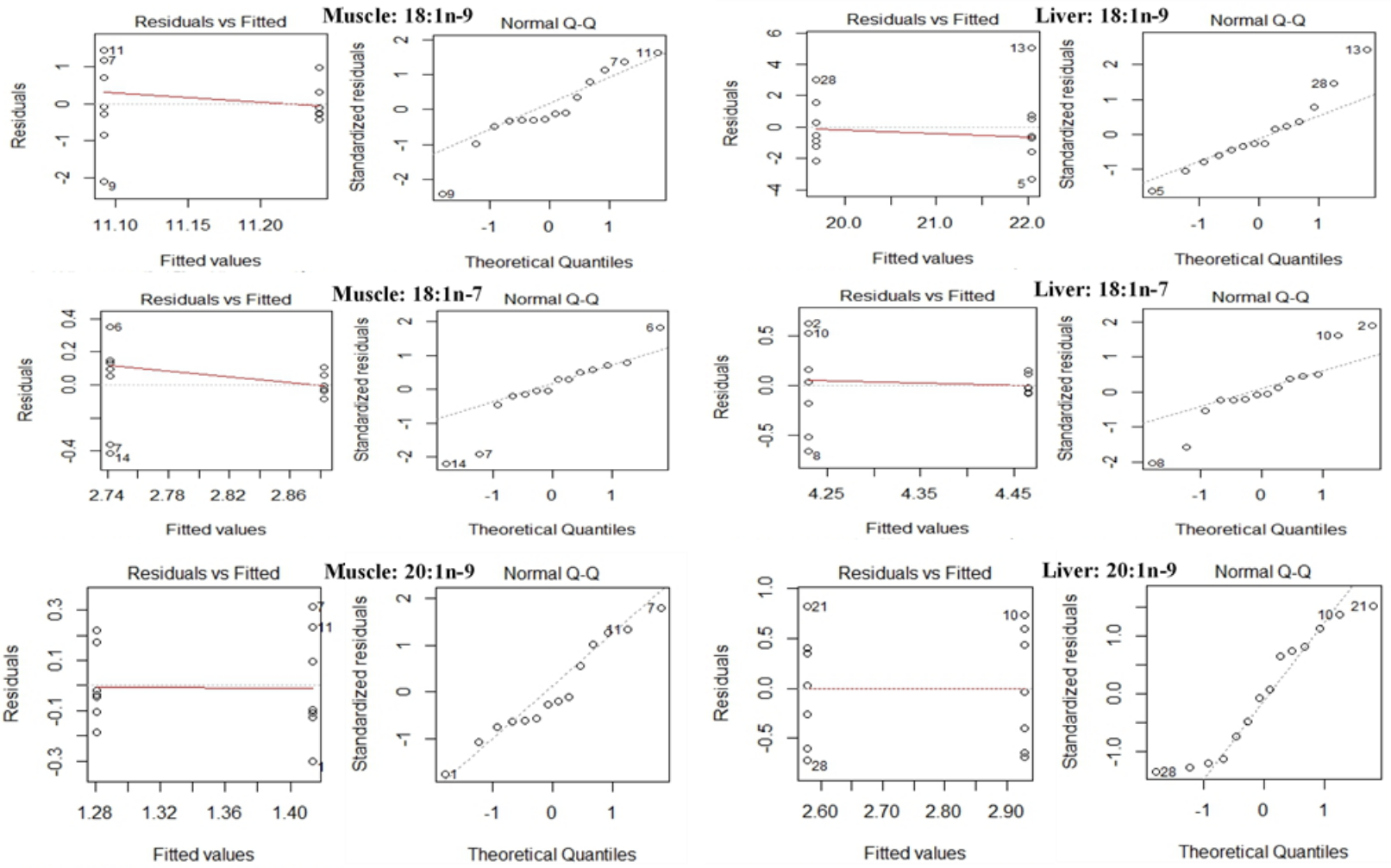
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:0
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: SFA
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: SFA
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 16:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 16:1n-7
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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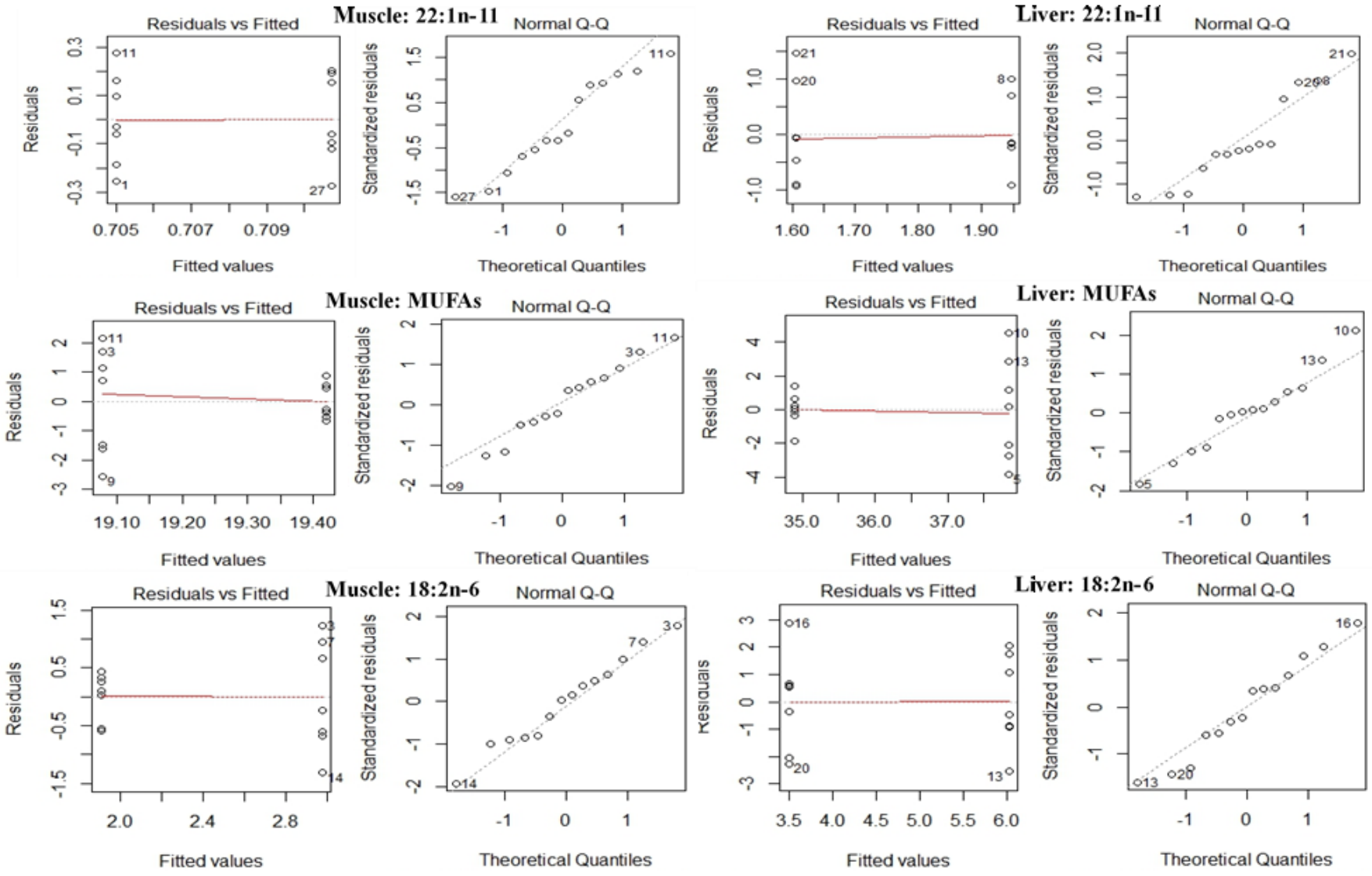
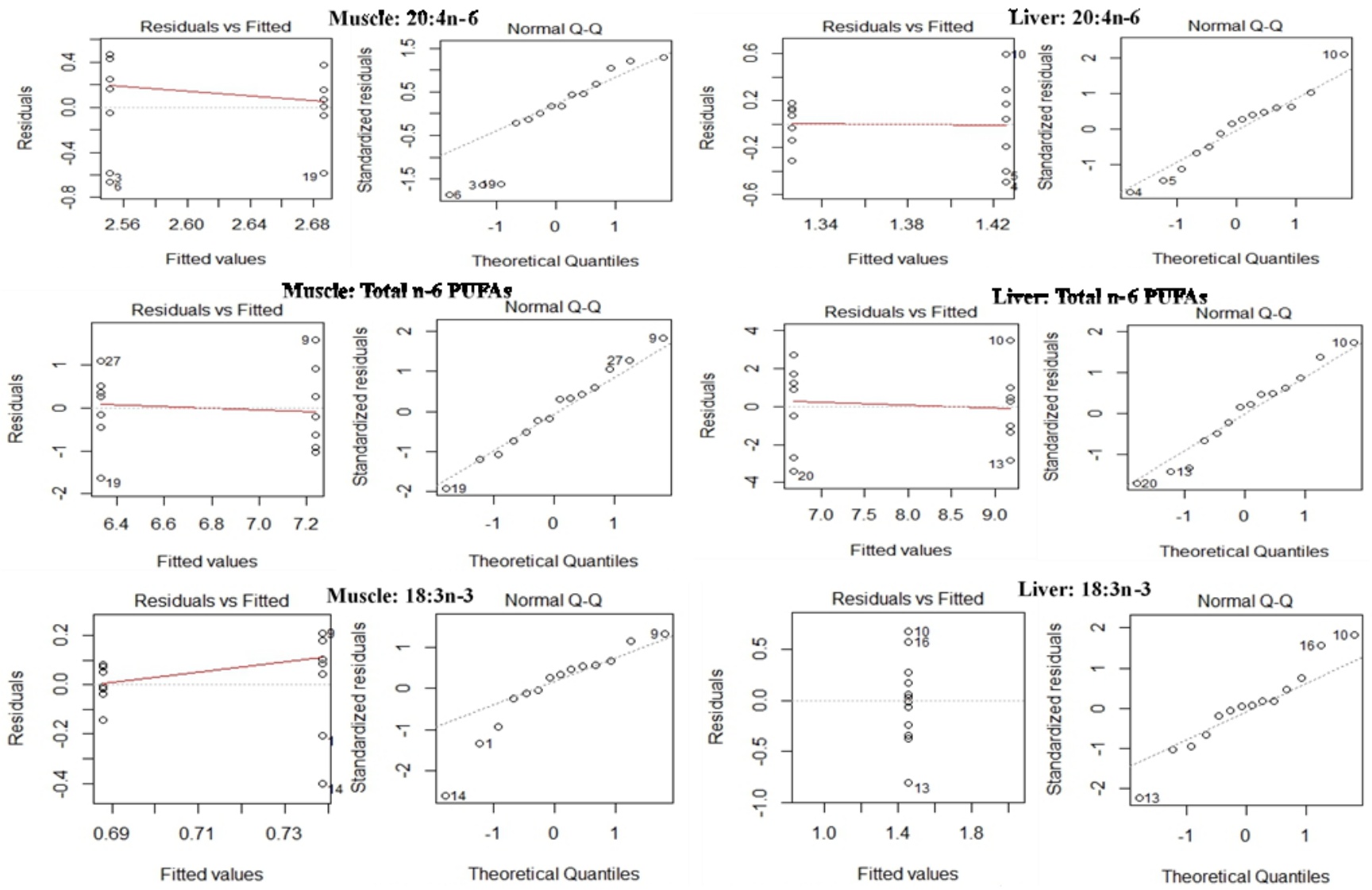
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles
Liver: 18:1n-9
Residuals vs Fitted
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles
Muscle: 18:1n-7
Residuals vs Fitted
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles
Liver: 18:1n-7
Residuals vs Fitted
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles
Muscle: 20:1n-9
Residuals vs Fitted
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles
Liver: 20:1n-9
Residuals vs Fitted
Normal Q-Q
Residuals
Fitted values
Standardized residuals
Theoretical Quantiles


Muscle: 22:1n-11
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:1n-11
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: MUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: MUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 18:2n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:2n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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Residuals
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Standardized residuals
Theoretical Quantiles
Liver: 20:4n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: Total n-6 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: Total n-6 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 18:3n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
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Liver: 18:3n-3
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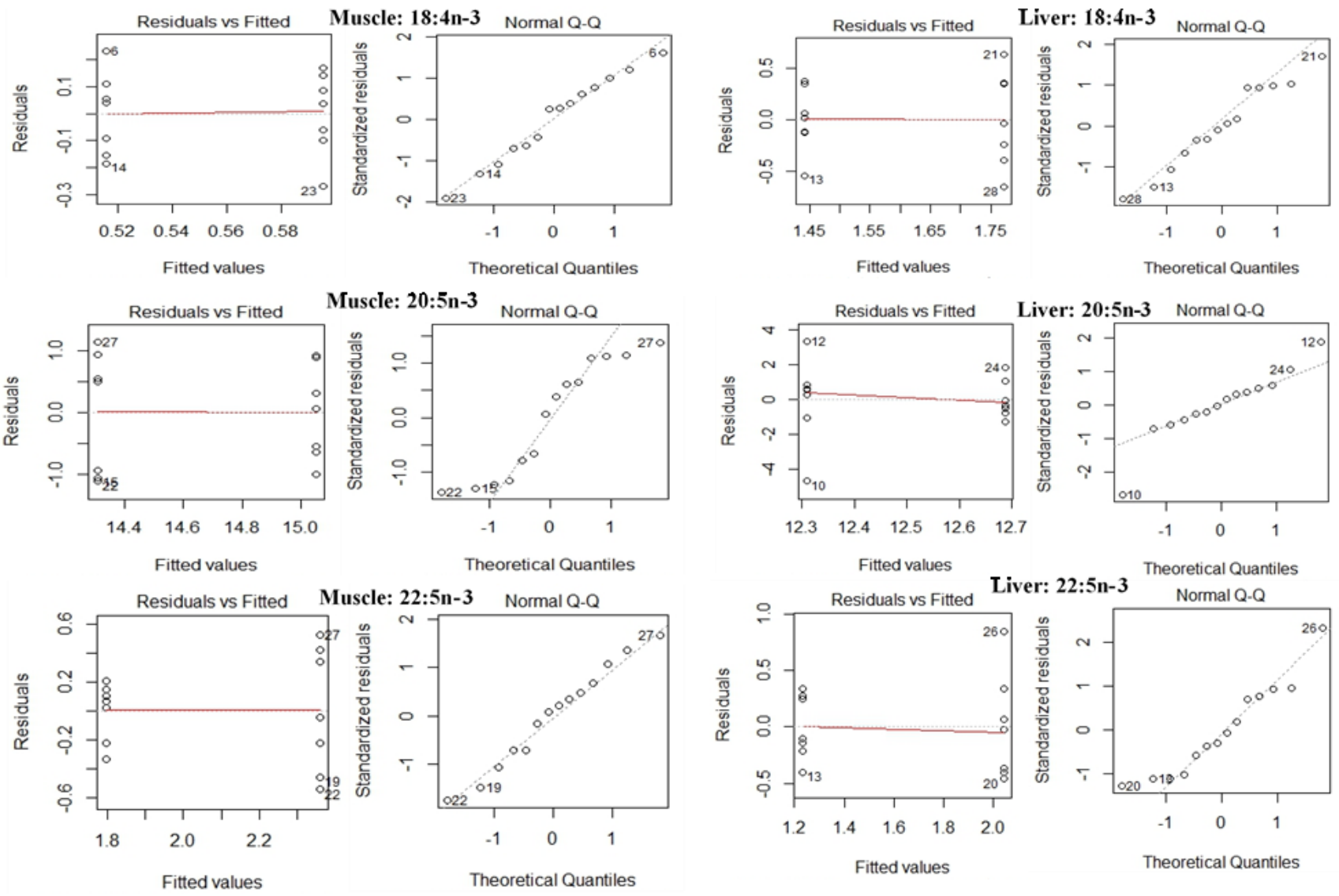
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles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Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 18:4n-3
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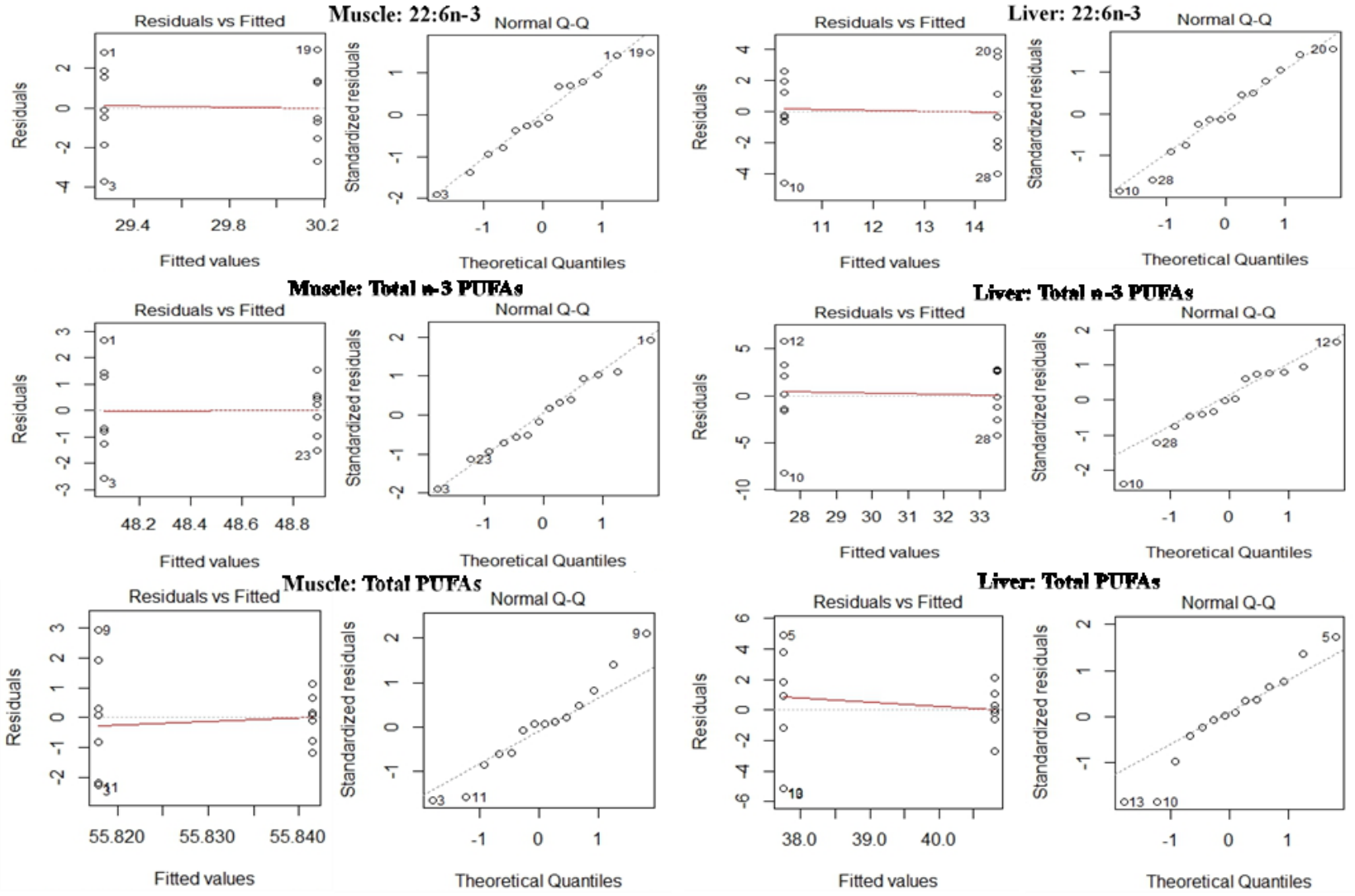
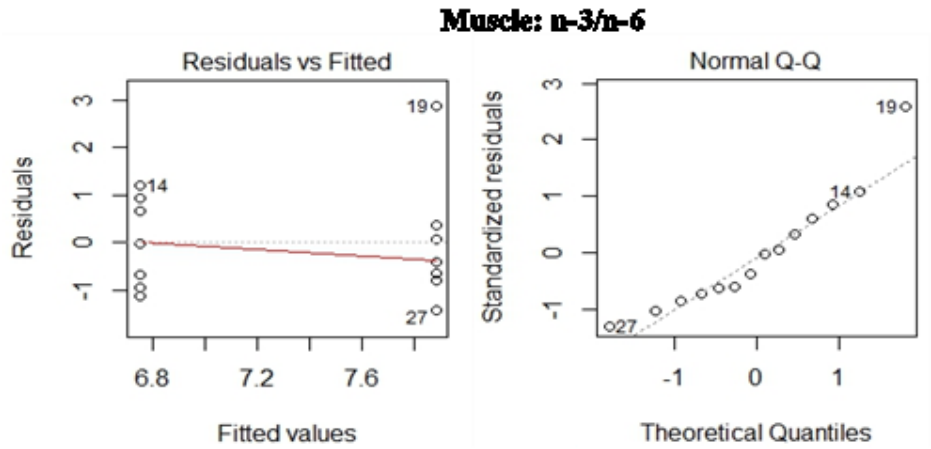
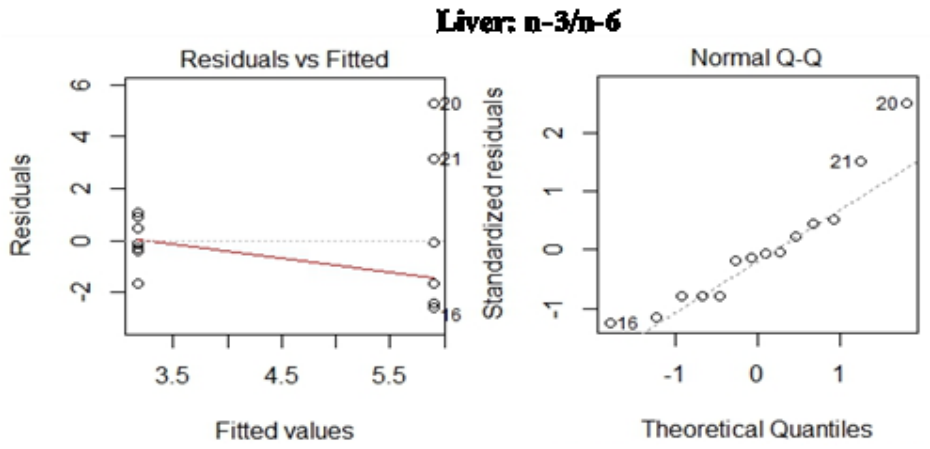
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 20:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 20:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: 22:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:5n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles


Muscle: 22:6n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: 22:6n-3
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: Total n-3 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: Total n-3 PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Muscle: Total PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles
Liver: Total PUFAs
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles


Muscle: n-3/n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles


Liver: n-3/n-6
Residuals vs Fitted
Residuals
Fitted values
Normal Q-Q
Standardized residuals
Theoretical Quantiles

APPENDIX C
ADDITIONAL INFORMATION FOR CHAPTER 5

The following appendix provides information on whiting sampled from Mallaig (Reference 3), and the full fatty (FA) profiles for commercial feeds and muscle tissues of mackerel and whiting caught near and away from two fish farms. Diagnostic plots for different models fit to the data for Chapter 5 are also included in this Appendix.

Table C.1 Length (cm), mass (g), FCI and HSI of whiting obtained from Mallaig (Reference 3). Data is presented as means and 95% confidence intervals.

	Reference 3
No. of fish	49
Length	27.8 [27.14, 28.50]
Mass	179.8 [165.86, 193.72]
FCI	0.82 [0.80, 0.84]
HSI	5.70 [5.21, 6.19]

Table C.2 Total lipid content (%) and fatty acid composition (%) of commercial diets used at Melfort and Leven farms. Data are presented as mean and 95% confidence intervals.

	Melfort Diet 2014	**Leven Diet 2014**
Total Lipid	25.58 [25.28, 25.88]	25.63 [23.67, 27.58]
Fatty Acids		
14:0	7.09 [6.77, 7.40]	3.27 [2.89, 3.65]
15:0	0.42 [0.29, 0.55]	0.26 [0.26, 0.26]
16:0	13.84 [7.49, 20.19]	11.92 [10.78, 13.06]
18:0	2.43 [2.23, 2.62]	3.33 [3.33, 3.33]
20:0	0.23 [0.23, 0.23]	0.44 [0.37, 0.50]
22:0	0.12 [0.12, 0.12]	0.23 [0.23, 0.23]
Total SFAs	21.99 [10.74, 33.23]	19.44 [17.92, 20.96]
16:1n-9	0.25 [0.05, 0.44]	0.09 [0.02, 0.15]
16:1n-7	4.56 [2.27, 6.85]	3.33 [2.95, 3.71]
18:1n-9	19.33 [14.63, 24.03]	36.63 [34.34, 38.92]
18:1n-7	2.91 [1.57, 4.24]	2.97 [2.84, 3.10]
20:1n-11	0.91 [0.66, 1.16]	0.06 [-0.70, 0.82]
20:1n-9	7.35 [6.52, 8.17]	1.72 [0.96, 2.48]
20:1n-7	0.28 [0.15, 0.41]	0.15 [0.15, 0.15]
22:1n-11	11.01 [9.54, 12.47]	0.93 [0.73, 1.12]
22:1n-9	1.06 [1.06, 1.06]	0.66 [0.59, 0.72]
24:1n-9	0.87 [0.74, 1.00]	0.32 [0.25, 0.38]
Total MUFAs	48.51 [45.33, 51.69]	46.84 [44.30, 49.38]
18:2n-6	7.38 [6.11, 8.65]	13.22 [11.88, 14.55]
18:3n-6	0.15 [-0.05, 0.34]	0.11 [0.11, 0.11]

20:2n-6	0.28 [0.03, 0.53]	0.12 [-0.01, 0.25]
20:3n-6	0.07 [0.07, 0.07]	0.07 [0.001, 0.13]
20:4n-6	0.45 [0.32, 0.58]	0.35 [0.28, 0.41]
22:4n-6	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]
22:5n-6	0.18 [0.05, 0.31]	0.13 [0.13, 0.13]
Total n-6 PUFA	8.50 [6.72, 10.28]	13.99 [12.85, 15.13]
18:3n-3	1.92 [0.96, 2.87]	5.14 [4.82, 5.45]
18:4n-3	2.05 [1.47, 2.62]	1.14 [1.01, 1.27]
20:3n-3	0.15 [0.02, 0.28]	0.04 [0.04, 0.04]
20:4n-3	0.63 [0.38, 0.88]	0.29 [0.16, 0.42]
20:5n-3	5.89 [4.42, 7.35]	5.93 [4.72, 7.13]
22:5n-3	0.99 [0.61, 1.37]	0.72 [0.52, 0.91]
22:6n-3	8.53 [5.99, 11.07]	4.79 [3.77, 5.81]
Total n-3 PUFA	20.16 [13.87, 26.44]	18.04 [15.18, 20.89]
16:2	0.30 [0.30, 0.30]	0.41 [0.41, 0.41]
16:3	0.19 [0.06, 0.32]	0.49 [0.42, 0.55]
16:4	0.36 [0.29, 0.42]	0.80 [0.73, 0.86]
Total 16:0	0.85 [0.72, 0.98]	1.69 [1.56, 1.82]
Total PUFAs	29.50 [21.50, 37.50]	33.72 [29.65, 37.79]
n-3/n-6	2.37 [2.14, 2.60]	1.29 [1.19, 1.39]

Table C.3 Total lipid content (%) and fatty acid concentration (%) in the muscle tissues of mackerel caught around two fish farms and three reference sites. Data are expressed as mean and 95% confidence intervals.

Location	**Melfort Farm**	**Leven Farm**	**Reference Mackerel 1 (=Isle of Luing)**	**Reference Mackerel 2 (=Oban Bay)**	**Reference Mackerel 3 (=Mallaig)**
No. of fish	22	17	17	17	17
Total Lipid	6.67 [4.23, 9.10]	7.17 [5.10, 9.24]	6.06 [4.27, 7.85]	6.93 [5.41, 8.46]	9.71 [7.15, 12.28]
Fatty Acids					
14:0	3.94 [3.55, 4.32]	3.57 [3.14, 4.00]	4.51 [4.24, 4.79]	3.58 [3.29, 3.88]	4.74 [4.38, 5.10]
15:0	0.50 [0.46, 0.54]	0.37 [0.34, 0.40]	0.67 [0.61, 0.72]	0.56 [0.52, 0.60]	0.67 [0.65, 0.69]
16:0	17.77 [17.13, 18.41]	17.81 [16.99, 18.63]	18.47 [17.93, 19.01]	19.66 [19.13, 20.18]	18.41 [17.73, 19.09]
18:0	4.44 [4.17, 4.71]	4.61 [4.25, 4.98]	4.43 [4.19, 4.66]	4.65 [4.48, 4.81]	4.14 [3.86, 4.41]
20:0	0.20 [0.17, 0.22]	0.16 [0.13, 0.19]	0.21 [0.18, 0.24]	0.16 [0.12, 0.19]	0.20 [0.16, 0.24]
22:0	0.08 [0.07, 0.09]	0.08 [0.07, 0.08]	0.10 [0.09, 0.11]	0.11 [0.10, 0.11]	0.10 [0.09, 0.11]
Total SFAs	26.92 [26.27, 27.57]	26.60 [25.65, 27.55]	28.38 [27.86, 28.91]	28.71 [28.26, 29.16]	28.25 [27.61, 28.90]
16:1n-9	0.22 [0.19, 0.25]	0.18 [0.15, 0.21]]	0.29 [0.25, 0.33]	0.24 [0.21, 0.28]	0.31 [0.28, 0.34]
16:1n-7	3.82 [3.59, 4.06]	4.04 [3.76, 4.32]	3.87 [3.65, 4.08]	3.99 [3.87, 4.11]	3.91 [3.75, 4.06]
18:1n-9	16.37 [14.48, 18.27]	18.61 [16.30, 20.92]	14.97 [13.80, 16.14]	19.34 [18.16, 20.51]	17.19 [15.61, 18.77]
18:1n-7	3.51 [3.20, 3.81]	3.75 [3.47, 4.02]	3.74 [3.51, 3.96]	4.54 [4.39, 4.70]	3.96 [3.67, 4.26]
20:1n-11	0.60 [0.52, 0.69]	0.34 [0.29, 0.38]	0.50 [0.42, 0.58]	0.39 [0.35, 0.44]	0.44 [0.38, 0.51]
20:1n-9	5.28 [4.62, 5.94]	4.50 [3.85, 5.16]	4.74 [4.18, 5.30]	3.86 [3.52, 4.21]	5.37 [4.71, 6.03]
20:1n-7	0.40 [0.34, 0.45]	0.39 [0.35, 0.43]	0.41 [0.32, 0.50]	0.31 [0.28, 0.34]	0.35 [0.30, 0.39]
22:1n-11	8.16 [6.62, 9.68]	6.00 [4.72, 7.28]	6.83 [5.56, 8.10]	4.94 [4.12, 5.76]	7.77 [6.45, 9.08]
22:1n-9	1.10 [0.93, 1.26]	1.10 [0.98, 1.22]	1.07 [0.82, 1.31]	0.76 [0.70, 0.82]	0.94 [0.87, 1.01]
24:1n-9	0.79 [0.74, 0.84]	0.78 [0.73, 0.82]	0.70 [0.67, 0.73]	0.71 [0.68, 0.74]	0.72 [0.66, 0.77]
Total MUFAs	40.23 [38.24, 42.22]	39.67 [37.59, 41.75]	37.11 [35.76, 38.45]	39.09 [38.35, 39.82]	40.94 [39.75, 42.14]
18:2n-6	1.90 [1.46, 2.33]	2.32 [1.46, 3.17]	1.34 [1.22, 1.46]	1.17 [1.08, 1.29]	1.45 [1.36, 1.52]
18:3n-6	0.15 [0.13, 0.16]	0.12 [0.10, 0.13]	0.16 [0.15, 0.18]	0.14 [0.13, 0.15]	0.17 [0.16, 0.18]
20:2n-6	0.29 [0.27, 0.31]	0.22 [0.21, 0.24]	0.31 [0.29, 0.33]	0.24 [0.22, 0.26]	0.31 [0.28, 0.33]
20:3n-6	0.07 [0.06, 0.08]	0.04 [0.03, 0.05]	0.09 [0.08, 0.10]	0.07 [0.06, 0.08]	0.09 [0.08, 0.10]

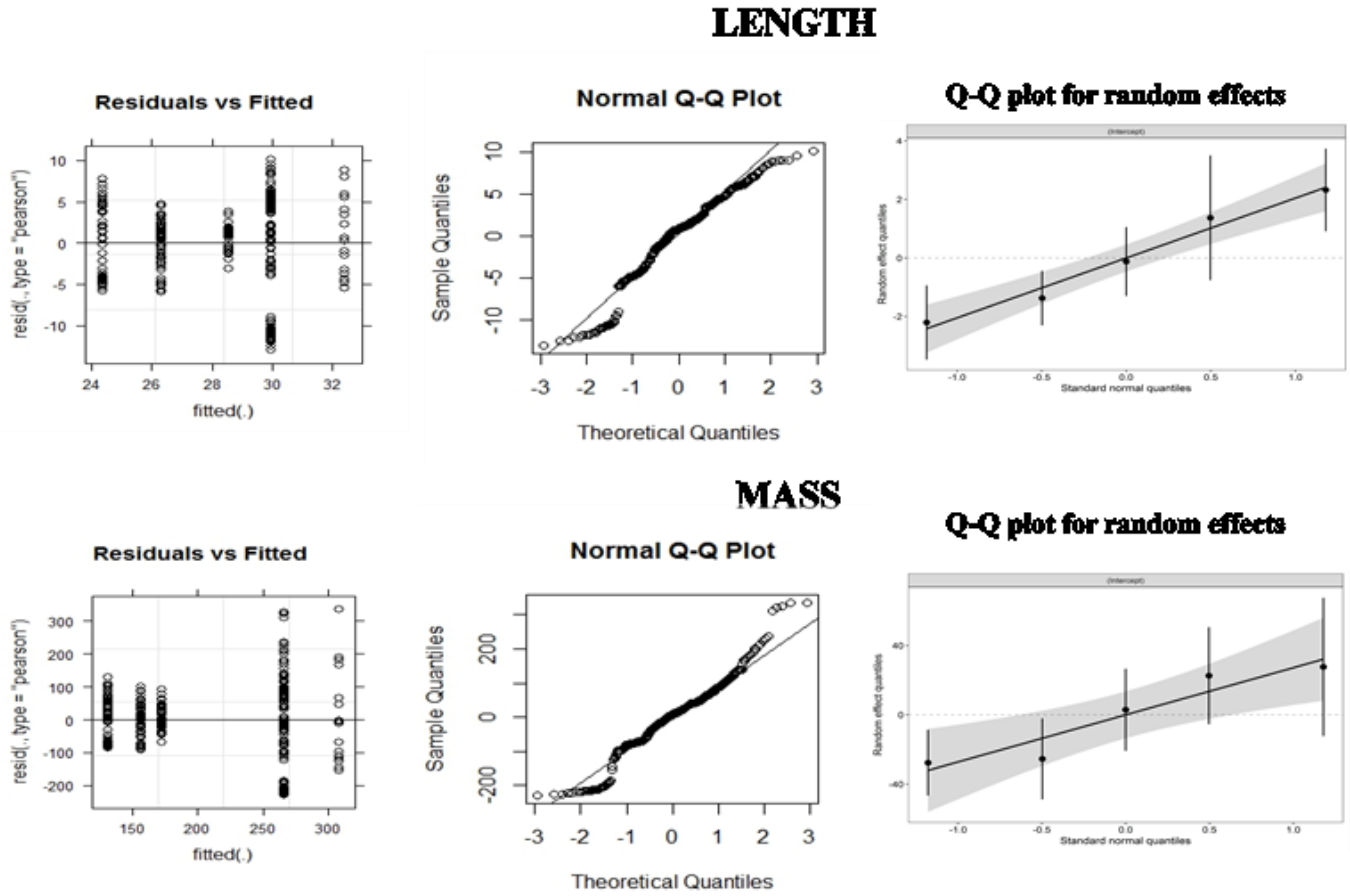
20:4n-6	1.15 [1.03, 1.28]	0.81 [0.70, 0.93]	1.16 [1.03, 1.29]	0.96 [0.88, 1.03]	1.10 [0.97, 1.23]
22:4n-6	0.24 [0.21, 0.27]	0.20 [0.15, 0.25]	0.26 [0.24, 0.29]	0.24 [0.21, 0.27]	0.19 [0.15, 0.22]
22:5n-6	0.40 [0.35, 0.45]	0.25 [0.20, 0.29]	0.38 [0.34, 0.42]	0.31 [0.28, 0.33]	0.38 [0.32, 0.43]
Total n-6 PUFA	4.20 [3.76, 4.65]	3.96 [3.16, 4.75]	3.71 [3.44, 3.97]	3.14 [2.97, 3.31]	3.66 [3.46, 3.87]
18:3n-3	1.00 [0.88, 1.11]	1.17 [0.91, 1.42]	0.97 [0.90, 1.04]	0.85 [0.78, 0.93]	1.05 [0.95, 1.14]
18:4n-3	1.69 [1.49, 1.89]	1.85 [1.54, 2.16]	2.02 [1.85, 2.18]	1.74 [1.58, 1.90]	2.19 [1.91, 2.47]
20:3n-3	0.21 [0.19, 0.23]	0.16 [0.14, 0.17]	0.25 [0.23, 0.27]	0.22 [0.20, 0.24]	0.26 [0.23, 0.28]
20:4n-3	0.70 [0.66, 0.74]	0.70 [0.65, 0.74]	0.80 [0.76, 0.84]	0.71 [0.68, 0.75]	0.80 [0.76, 0.84]
20:5n-3	6.54 [6.13, 6.95]	8.09 [7.61, 8.58]	7.06 [6.66, 7.46]	7.20 [6.86, 7.54]	6.03 [5.71, 6.34]
22:5n-3	1.57 [1.42, 1.72]	1.63 [1.55, 1.71]	1.52 [1.44, 1.59]	1.53 [1.47, 1.58]	1.32 [1.26, 1.39]
22:6n-3	15.80 [14.30, 17.31]	15.09 [13.21, 16.98]	16.98 [16.06, 17.90]	15.73 [15.18, 16.27]	14.35 [13.72, 14.99]
Total n-3 PUFA	27.52 [25.89, 29.15]	28.69 [26.90, 30.48]	29.60 [28.52, 30.67]	27.99 [27.24, 28.74]	26.00 [25.08, 26.92]
16:2	0.58 [0.54, 0.62]	0.41 [0.39, 0.44]	0.62 [0.58, 0.66]	0.52 [0.49, 0.56]	0.56 [0.53, 0.58]
16:3	0.33 [0.31, 0.36]	0.32 [0.29, 0.34]	0.43 [0.39, 0.46]	0.41 [0.39, 0.42]	0.43 [0.42, 0.45]
16:4	0.21 [0.18, 0.24]	0.36 [0.30, 0.41]	0.17 [0.12, 0.21]	0.15 [0.12, 0.18]	0.15 [0.13, 0.17]
Total	1.13 [1.07, 1.18]	1.09 [1.02, 1.16]	1.28 [1.16, 1.26]	1.08 [1.04, 1.11]	1.14 [1.11, 1.17]
Total PUFA	32.85 [31.19, 34.51]	33.73 [32.24, 35.21]	34.51 [33.43, 35.59]	32.20 [31.41, 33.00]	30.81 [29.82, 31.79]
n-3/n-6	7.36 [6.53, 8.20]	9.23 [7.92, 10.54]	8.37 [7.63, 9.11]	9.13 [8.58, 9.69]	7.23 [6.83, 7.64]

Table C.4 Total lipid content (%) and fatty acid concentration (%) in whiting caught around two fish farms and two reference sites. Data are presented as means and 95% confidence intervals.

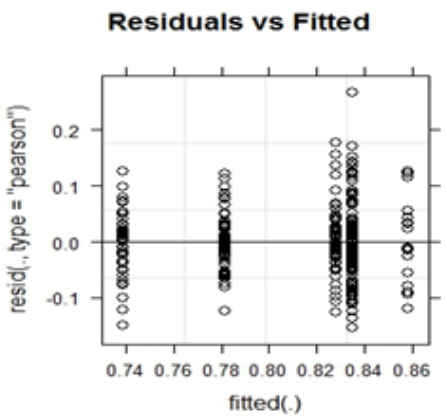
	Melfort Farm	**Leven Farm**	**Reference Whiting (=Firth of Clyde)**	**Reference Whiting (=North Minch)**	**Reference Whiting (=Mallaig)**
No. of fish	19	17	19	17	17
Total Lipid	1.13 [1.01, 1.24]	1.01 [0.88, 1.14]	1.00 [0.90, 1.09]	1.01 [0.92, 1.09]	0.92 [0.84, 1.01]
Fatty Acids					
14:0	1.05 [0.98, 1.13]	1.32 [1.20, 1.43]	1.00 [0.93, 1.06]	0.95 [0.89, 1.01]	1.41 [1.33, 1.48]
15:0	0.37 [0.34, 0.39]	0.38 [0.36, 0.41]	0.37 [0.34, 0.39]	0.38 [0.36, 0.40]	0.31 [0.30, 0.32]
16:0	15.84 [15.42, 16.39]	16.93 [16.38, 17.48]	18.38 [18.08, 18.85]	17.01 [16.59, 17.57]	19.24 [19.00, 19.48]
18:0	5.75 [5.49, 5.98]	5.98 [5.74, 6.22]	5.89 [5.77, 5.99]	5.82 [5.67, 5.98]	5.25 [5.10, 5.39]
20:0	0.10 [0.09, 0.11]	0.08 [0.07, 0.09]	0.08 [0.06, 0.09]	0.03 [0.02, 0.04]	0.06 [0.05, 0.07]
22:0	0.01 [0.01, 0.03]	0.02 [0.01, 0.03]	0.03 [0.02, 0.04]	0.01 [0.00, 0.02]	0.02 [0.01, 0.03]
Total SFAs	23.12 [22.53, 23.83]	24.71 [24.07, 25.35]	25.74 [25.39, 26.23]	24.20 [23.68, 24.87]	26.28 [25.98, 26.59]
16:1n-9	0.20 [0.16, 0.23]	0.33 [0.27, 0.39]	0.29 [0.25, 0.34]	0.28 [0.23, 0.35]	0.20 [0.16, 0.25]
16:1n-7	2.08 [1.98, 2.19]	1.91 [1.79, 2.03]	1.83 [1.70, 1.96]	1.34 [1.28, 1.39]	2.35 [2.23, 2.46]
18:1n-9	11.12 [9.99, 12.06]	10.62 [9.66, 11.58]	8.23 [7.88, 8.63]	7.59 [7.21, 7.99]	9.67 [9.32, 10.01]
18:1n-7	3.25 [3.15, 3.36]	3.41 [3.31, 3.51]	3.18 [3.04, 3.35]	2.57 [2.38, 2.81]	2.57 [2.46, 2.68]
20:1n-11	0.38 [0.33, 0.45]	0.63 [0.53, 0.74]	0.19 [0.16, 0.21]	0.34 [0.31, 0.36]	0.53 [0.50, 0.55]
20:1n-9	1.63 [1.42, 1.83]	1.29 [1.14, 1.44]	0.85 [0.76, 0.90]	1.35 [1.22, 1.43]	2.20 [2.06, 2.33]
20:1n-7	0.49 [0.37, 0.51]	0.44 [0.37, 0.51]	0.18 [0.16, 0.20]	0.12 [0.10, 0.14]	0.13 [0.12, 0.15]
22:1n-11	0.91 [0.73, 1.10]	0.52 [0.43, 0.61]	0.44 [0.36, 0.49]	0.57 [0.43, 0.68]	1.40 [1.22, 1.57]
22:1n-9	0.34 [0.29, 0.39]	0.27 [0.23, 0.31]	0.23 [0.20, 0.26]	0.25 [0.21, 0.28]	0.26 [0.24, 0.27]
24:1n-9	0.46 [0.44, 0.48]	0.39 [0.35, 0.43]	0.63 [0.58, 0.66]	0.67 [0.63, 0.70]	0.70 [0.67, 0.73]
Total MUFAs	20.84 [19.50, 22.02]	19.82 [18.57, 21.07]	16.04 [15.44, 16.64]	15.08 [14.43, 15.73]	20.00 [19.32, 20.67]
18:2n-6	2.84 [2.30, 3.31]	2.84 [2.24, 3.44]	1.15 [1.09, 1.24]	0.76 [0.72, 0.81]	0.69 [0.65, 0.74]
18:3n-6	0.15 [0.14, 0.17]	0.17 [0.15, 0.19]	0.12 [0.11, 0.14]	0.13 [0.12, 0.14]	0.11 [0.09, 0.12]
20:2n-6	0.39 [0.36, 0.42]	1.00 [0.81, 1.17]	0.25 [0.24, 0.27]	0.27 [0.26, 0.29]	0.19 [0.18, 0.20]

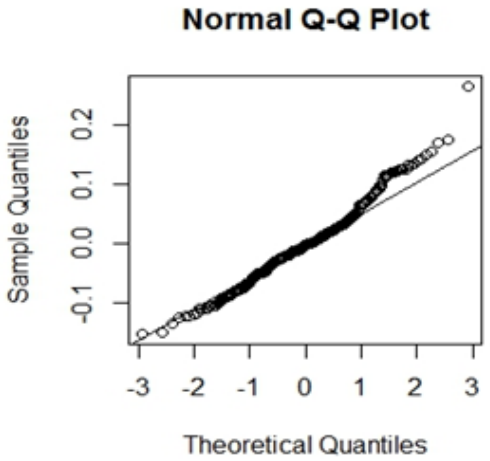
20:3n-6	0.07 [0.05, 0.08]	0.31 [0.24, 0.37]	0.07 [0.04, 0.09]	0.08 [0.05, 0.11]	0.08 [0.06, 0.10]
20:4n-6	2.50 [2.33, 2.55]	2.41 [2.26, 2.55]	1.78 [1.56, 1.92]	1.84 [1.65, 1.96]	2.18 [2.04, 2.32]
22:4n-6	0.25 [0.20, 0.29]	0.41 [0.35, 0.47]	0.10 [0.07, 0.13]	0.09 [0.06, 0.12]	0.15 [0.12, 0.18]
22:5n-6	0.42 [0.39, 0.44]	0.37 [0.33, 0.40]	0.47 [0.42, 0.50]	0.63 [0.54, 0.68]	0.46 [0.43, 0.49]
Total n-6 PUFA	6.62 [6.06, 7.07]	7.50 [6.63, 8.37]	3.95 [3.70, 4.11]	3.80 [3.47, 4.02]	3.85 [3.66, 4.03]
18:3n-3	0.75 [0.64, 0.85]	0.96 [0.80, 1.12]	0.61 [0.58, 0.65]	0.38 [0.35, 0.40]	0.36 [0.34, 0.38]
18:4n-3	0.51 [0.47, 0.58]	0.48 [0.43, 0.53]	0.99 [0.92, 1.09]	0.45 [0.43, 0.48]	0.75 [0.70, 0.81]
20:3n-3	0.13 [0.12, 0.14]	0.33 [0.26, 0.40]	0.21 [0.19, 0.23]	0.15 [0.14, 0.16]	0.11 [0.11, 0.12]
20:4n-3	0.43 [0.41, 0.45]	0.60 [0.57, 0.63]	0.56 [0.54, 0.58]	0.49 [0.45, 0.52]	0.56 [0.54, 0.58]
20:5n-3	12.85 [12.30, 13.45]	15.19 [14.58, 15.80]	14.25 [13.44, 15.17]	10.83 [10.50, 11.25]	12.96 [12.48, 13.43]
22:5n-3	2.32 [2.16, 2.44]	2.79 [2.54, 3.04]	1.22 [1.15, 1.26]	1.23 [1.14, 1.31]	1.60 [1.54, 1.67]
22:6n-3	31.24 [30.21, 32.41]	26.64 [24.28, 29.00]	35.35 [34.06, 36.47]	42.27 [41.35, 43.07]	32.67 [31.76, 33.58]
Total n-3 PUFA	48.24 [47.03, 49.59]	46.99 [45.10, 48.88]	53.19 [52.20, 54.14]	55.80 [54.75, 56.81]	49.01 [48.14, 49.89]
16:2	0.82 [0.74, 0.91]	0.55 [0.45, 0.65]	0.68 [0.61, 0.76]	0.65 [0.59, 0.70]	0.49 [0.46, 0.52]
16:3	0.22 [0.20, 0.24]	0.27 [0.23, 0.31]	0.24 [0.22, 0.26]	0.29 [0.28, 0.31]	0.19 [0.17, 0.20]
16:4	0.14 [0.13, 0.15]	0.16 [0.15, 0.17]	0.15 [0.14, 0.16]	0.18 [0.17, 0.19]	0.18 [0.16, 0.20]
Total 16	1.18 [1.08, 1.28]	0.98 [0.88, 1.08]	1.08 [0.99, 1.16]	1.12 [1.06, 1.18]	0.86 [0.83, 0.89]
Total PUFA	56.04 [55.02, 57.10]	55.47 [54.16, 56.77]	58.22 [57.24, 59.07]	60.72 [59.53, 61.76]	53.72 [52.85, 54.59]
n-3/n-6	7.85 [7.02, 8.84]	7.50 [6.10, 8.91]	13.87 [13.14, 14.84]	15.23 [14.46, 16.34]	13.01 [12.26, 13.77]

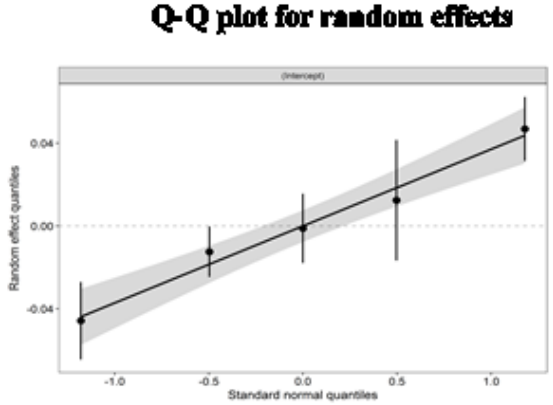
Figure C.1 Residual plots for mixed effect models for length, mass, FCI, and HSI for mackerel sampled near and away from two fish farms and three reference sites.



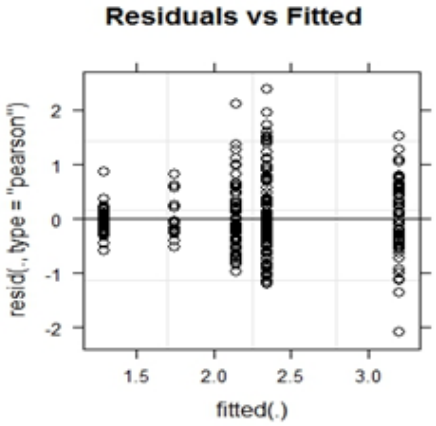
FCI

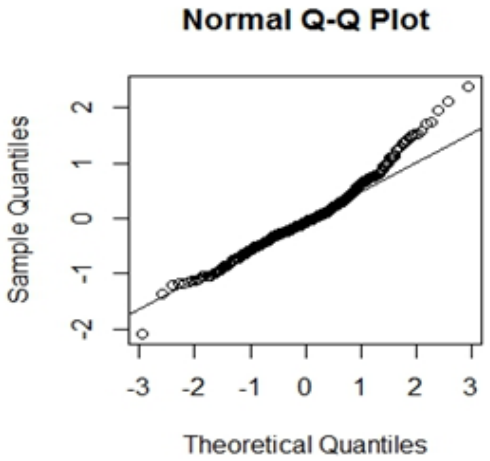

Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles


Q-Q plot for random effects
(Intercept)
Random effect quantiles
Standard normal quantiles

HSI


Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles

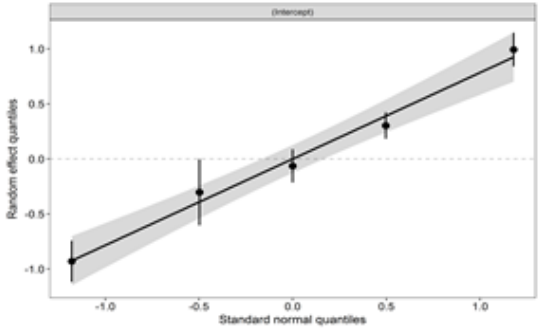
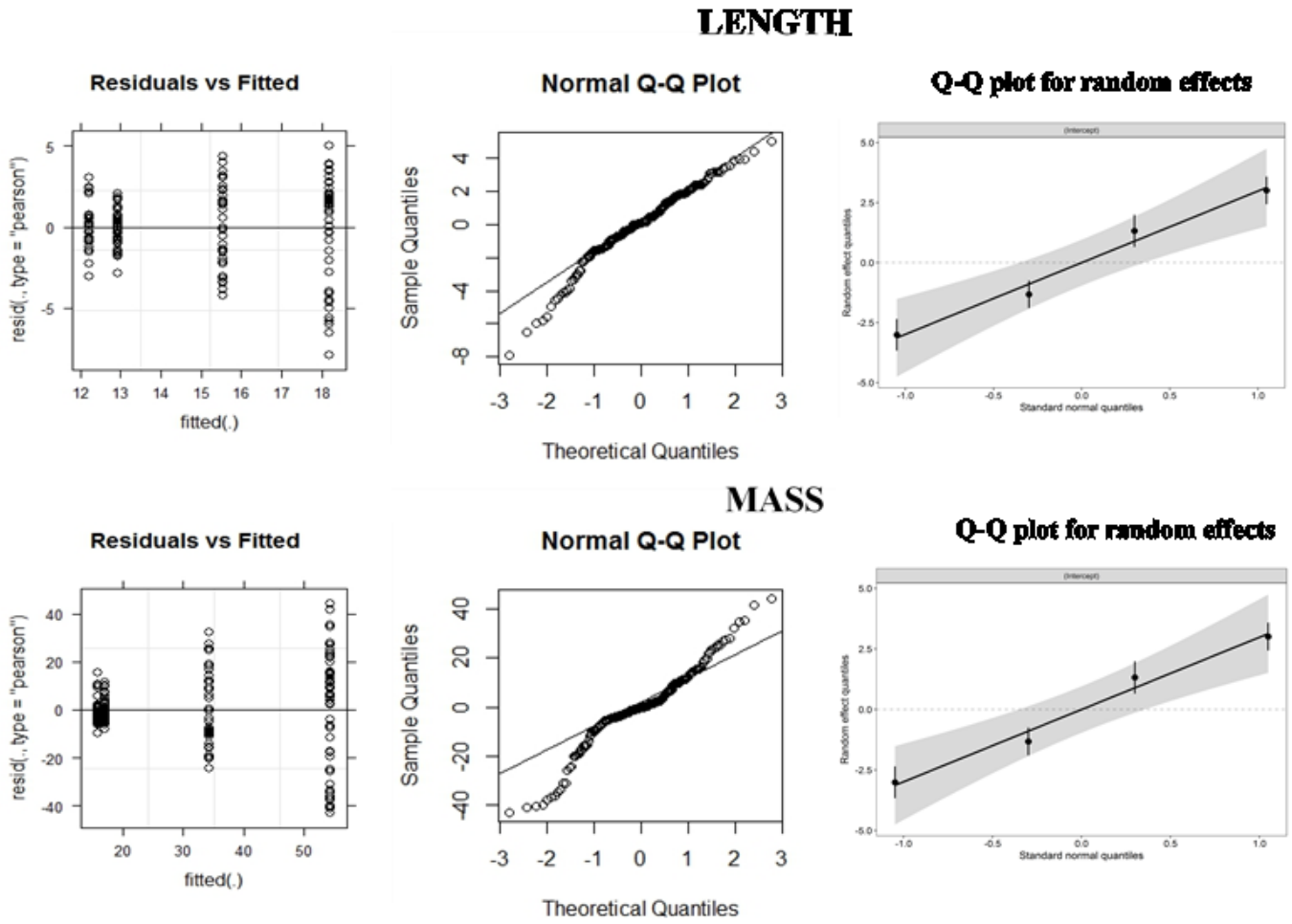
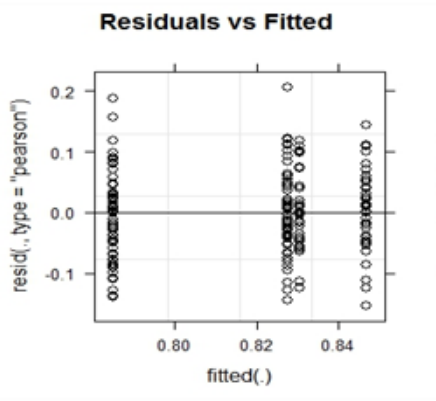
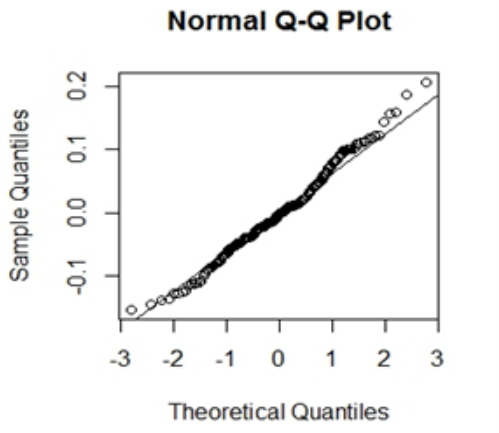

Q-Q plot for random effects
(Intercept)
Random effect quantiles
Standard normal quantiles

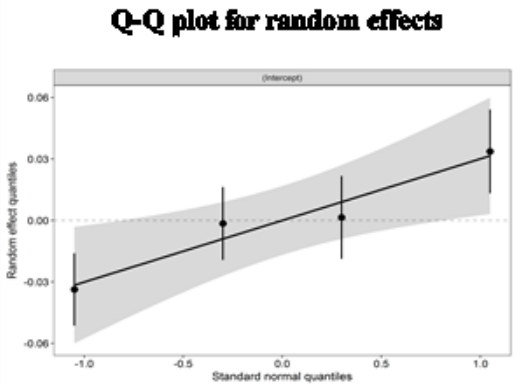
Figure C.2 Residual plots for mixed effect models for length, mass, FCI, and HSI for whiting sampled near and away from two fish farms and two reference sites.



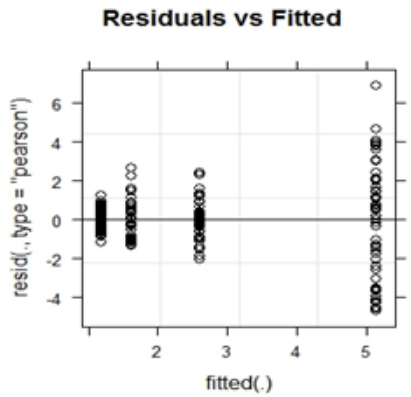
FCI

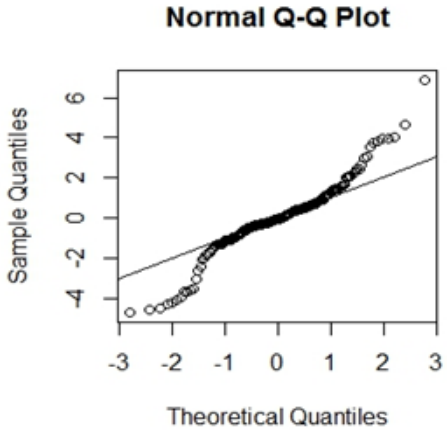

Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles


Q-Q plot for random effects
(Intercept)
Random effect quantiles
Standard normal quantiles

HSI


Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles

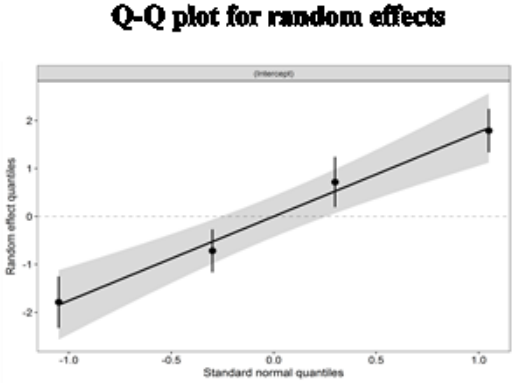
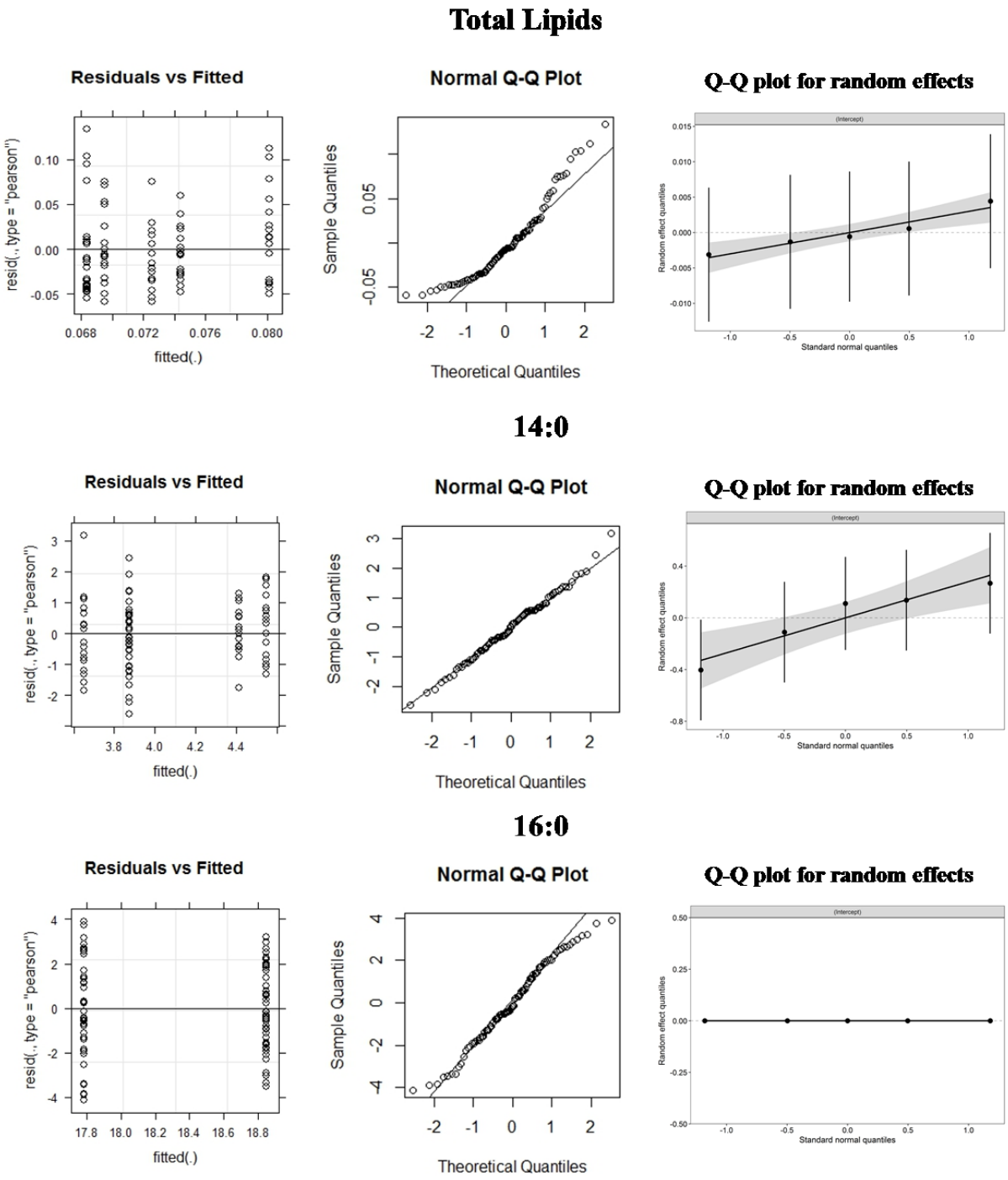

Q-Q plot for random effects
(Intercept)
Random effect quantiles
Standard normal quantiles

Figure C.3 Residual plots for mixed effect models for total lipid and selected fatty acids for muscle tissues of mackerel sampled near and away from two fish farms and two reference sites.



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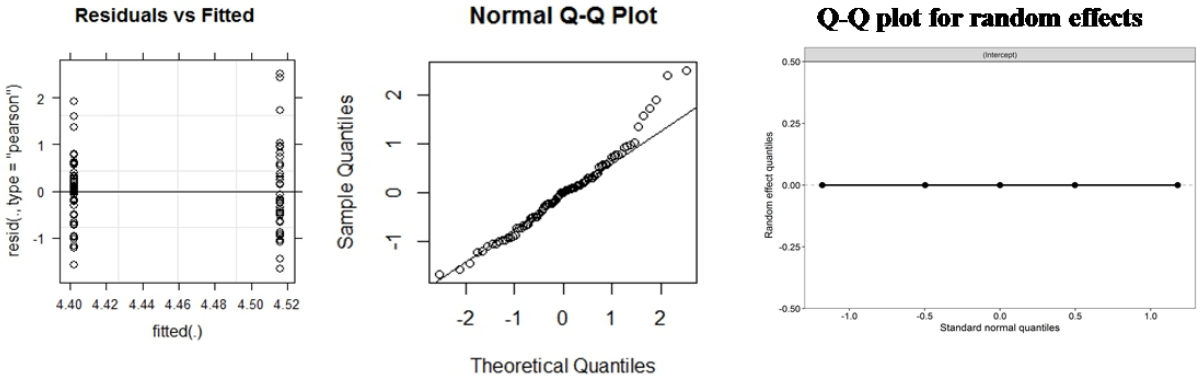
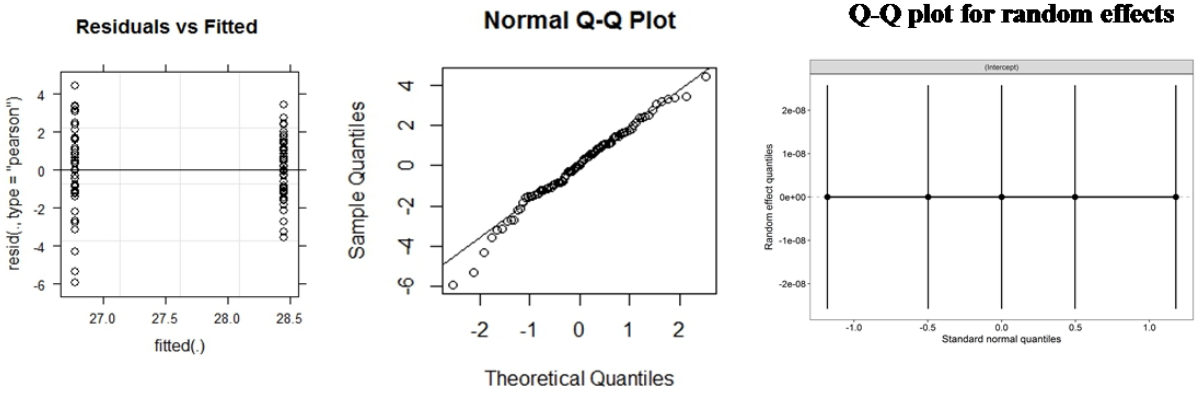


SFA



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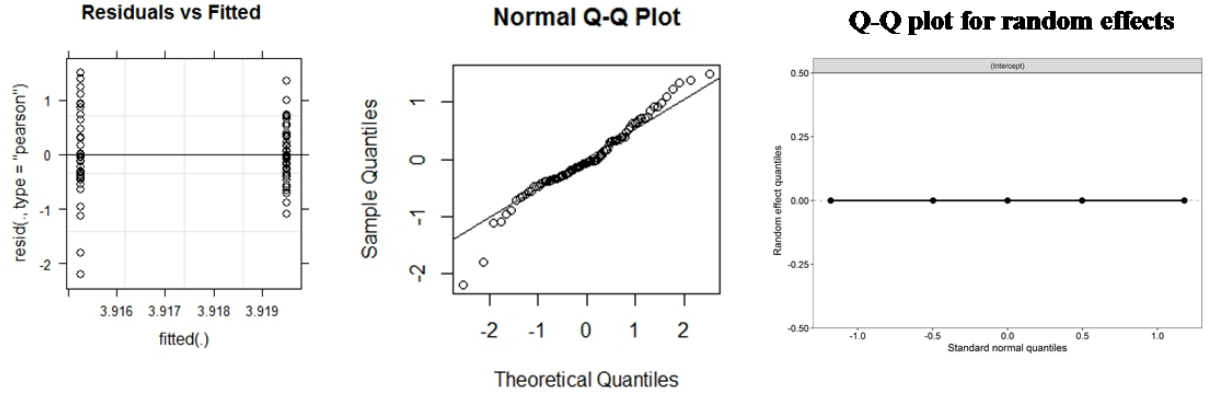
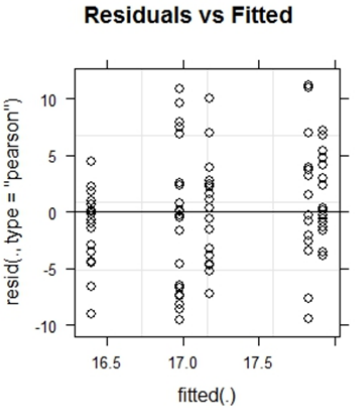
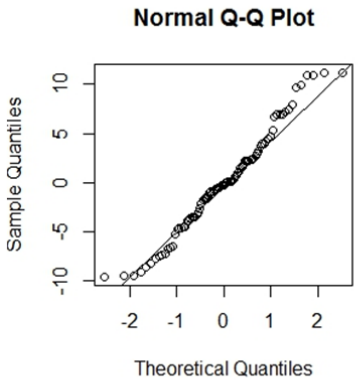
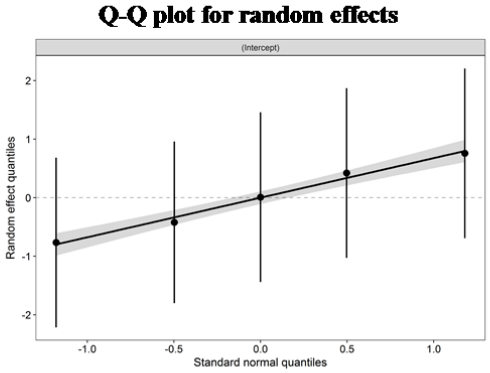


18:1n-9







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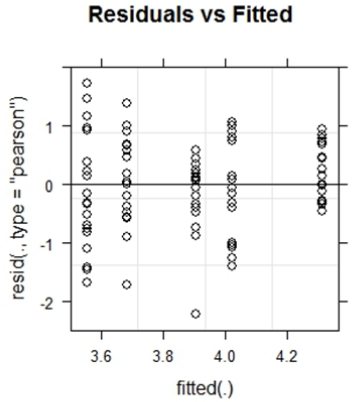

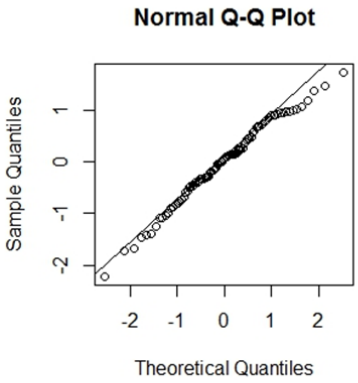

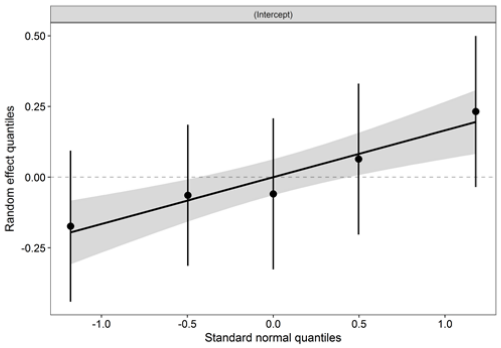

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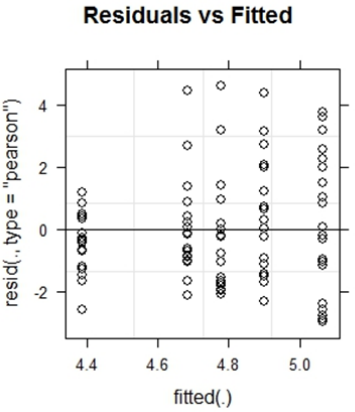

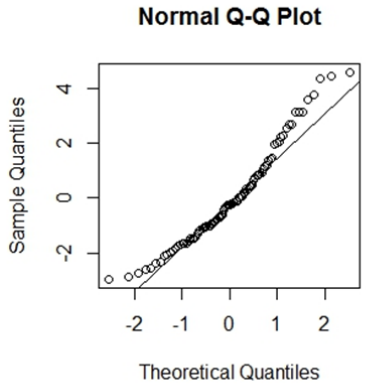

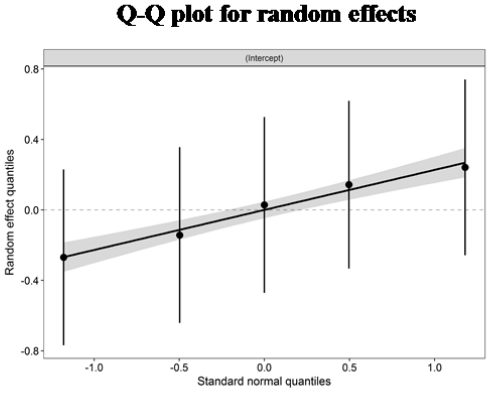

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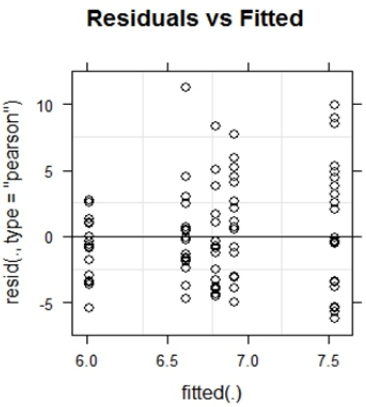
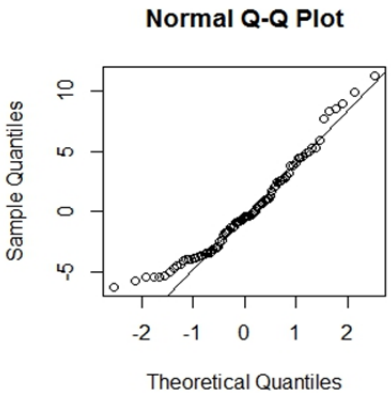
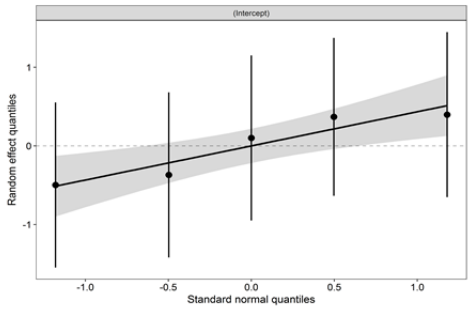
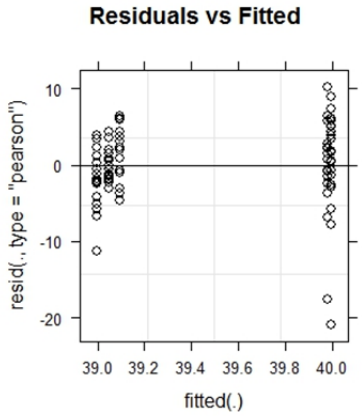
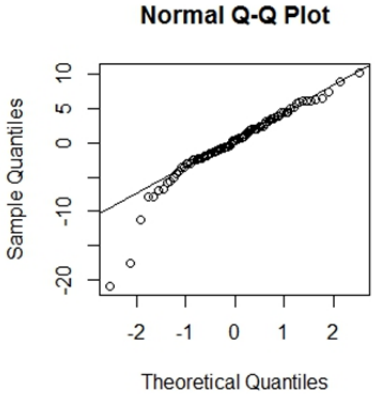


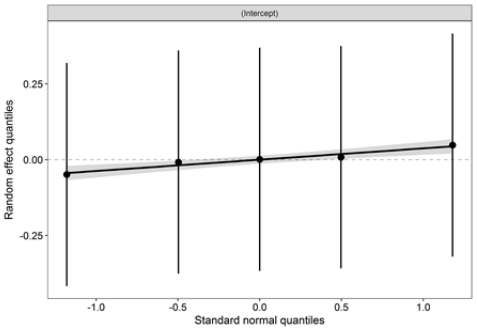




MUFAs







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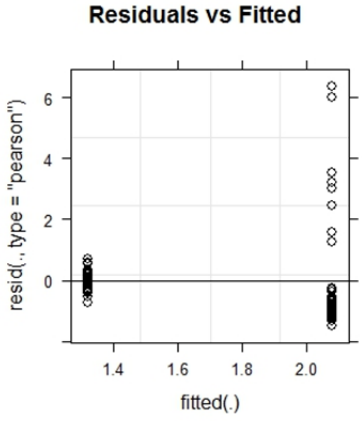

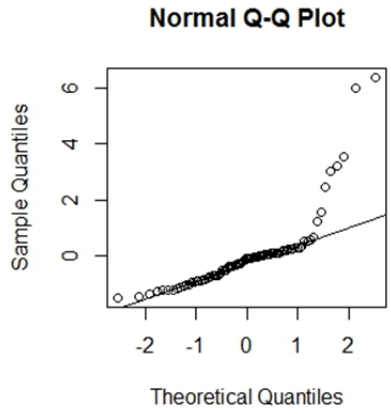

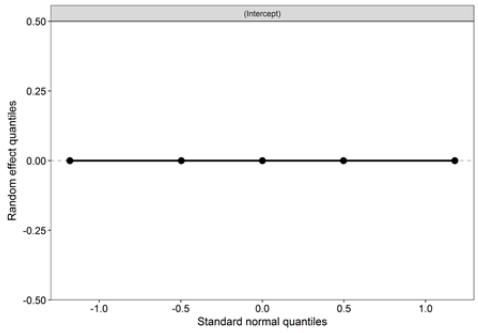

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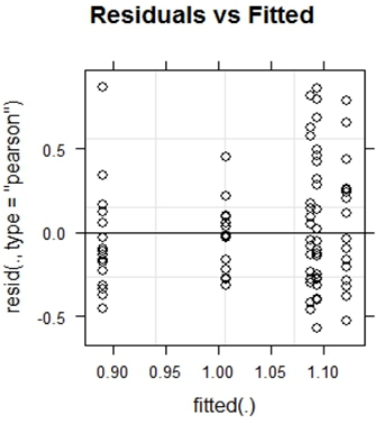
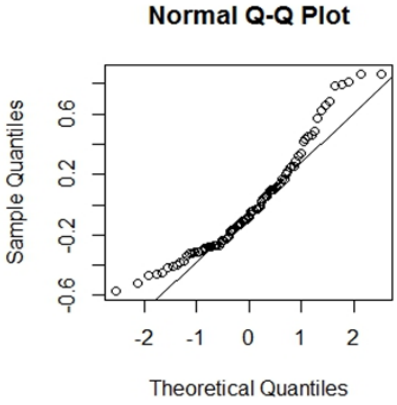
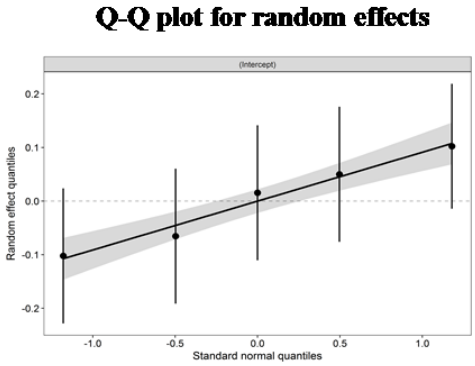
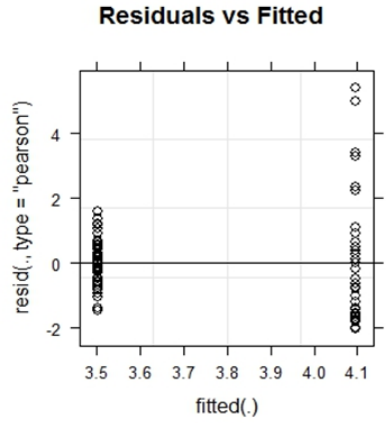
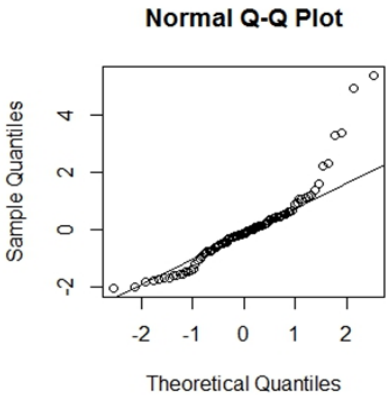


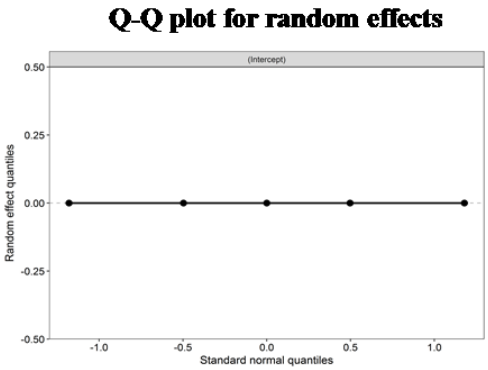




Total 6-PUFAs







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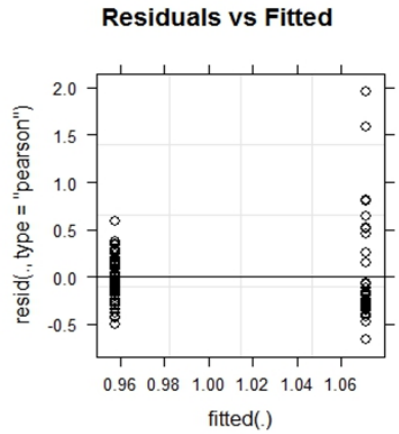

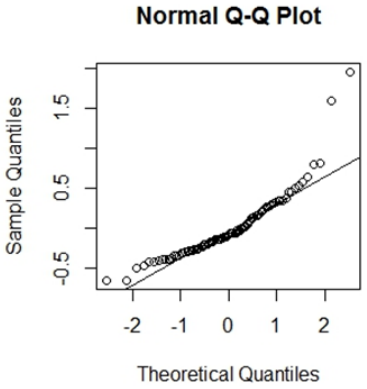

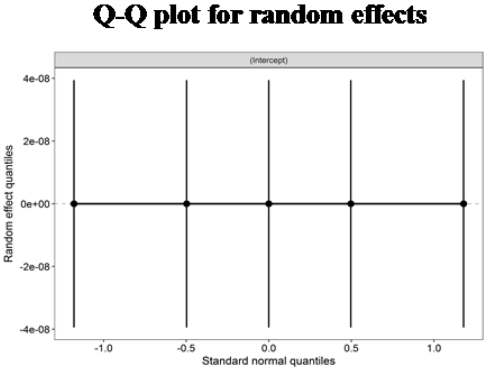

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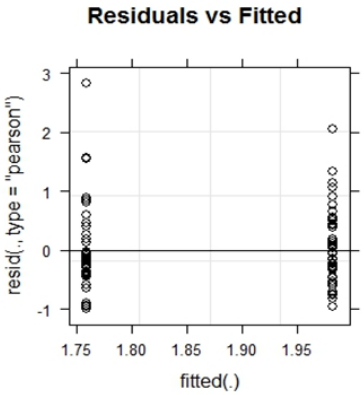

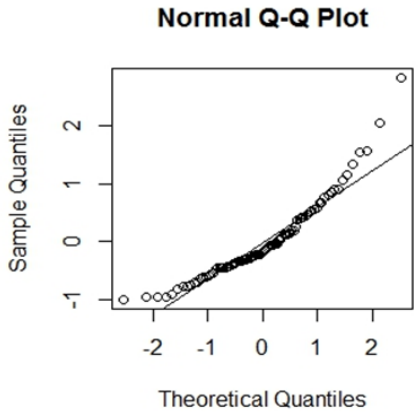

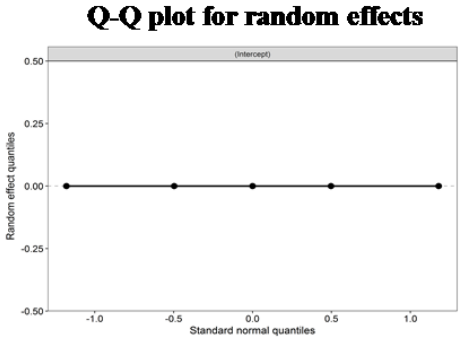

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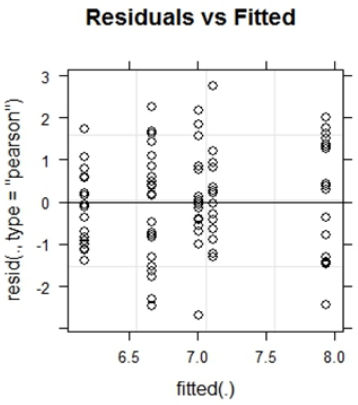

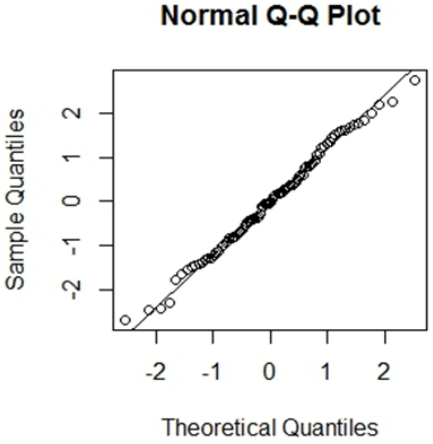

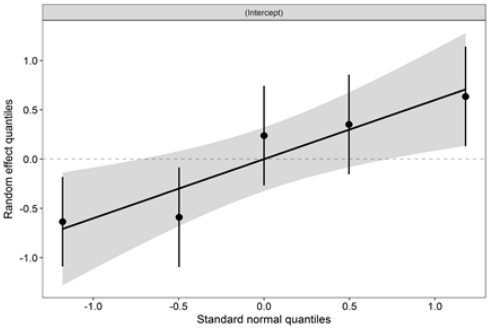

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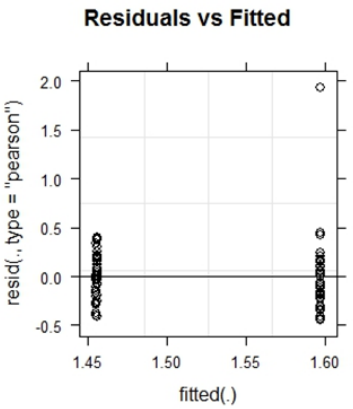
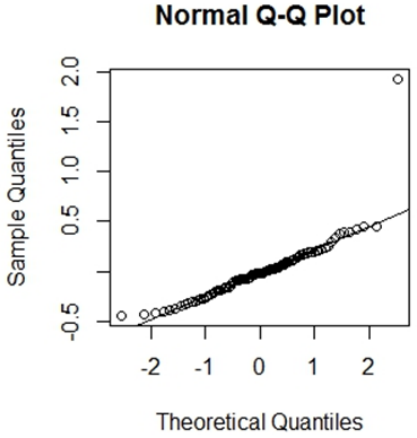
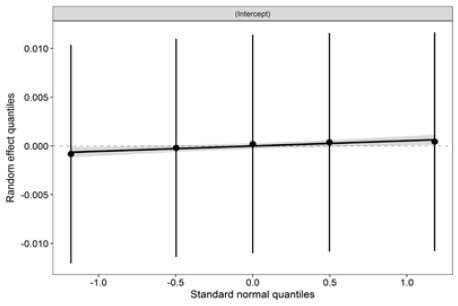
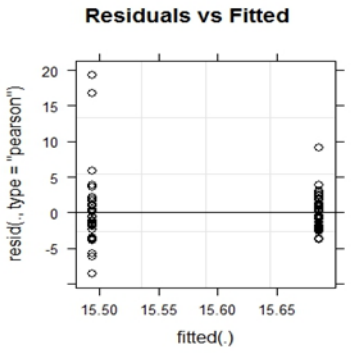
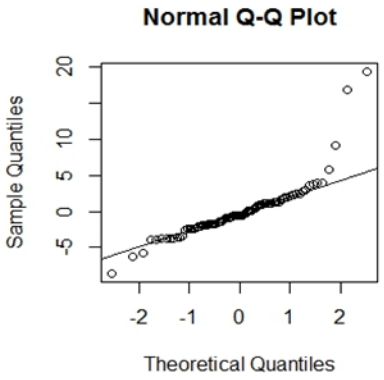


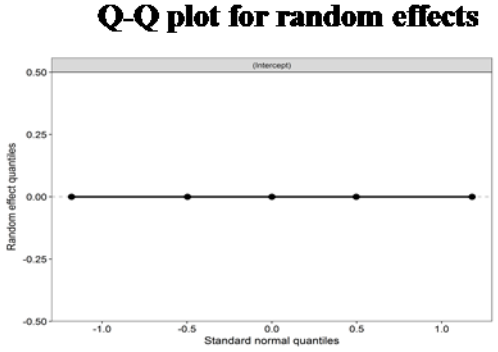




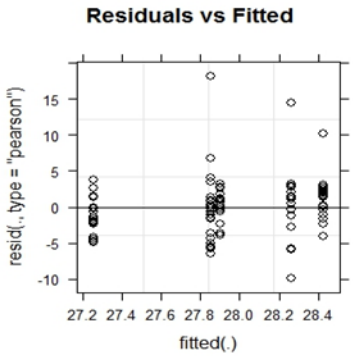
18:4n-3

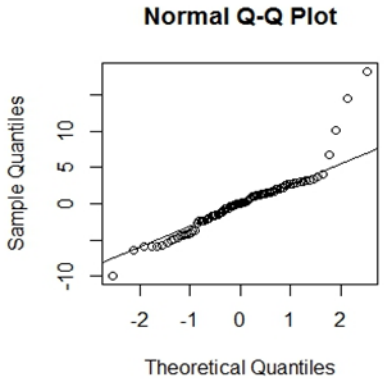


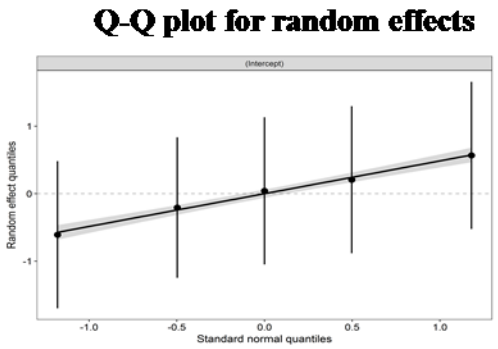




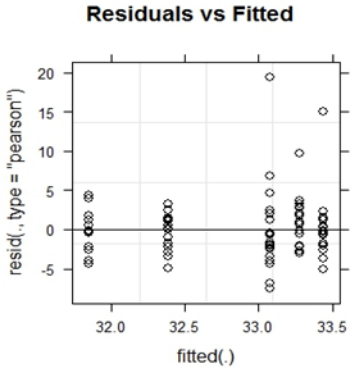
Total n-3 PUFAs

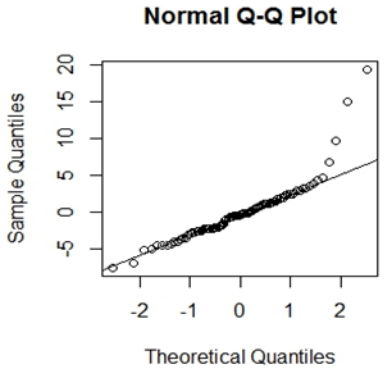


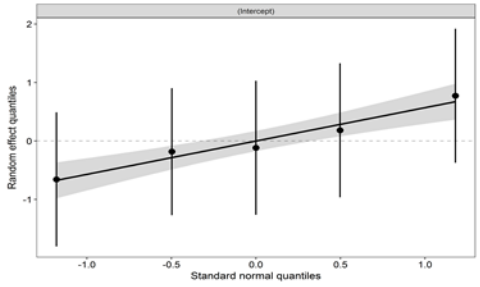




Total PUFAs







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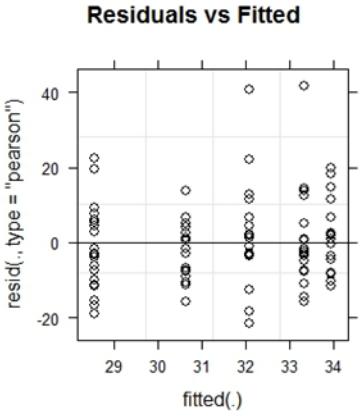
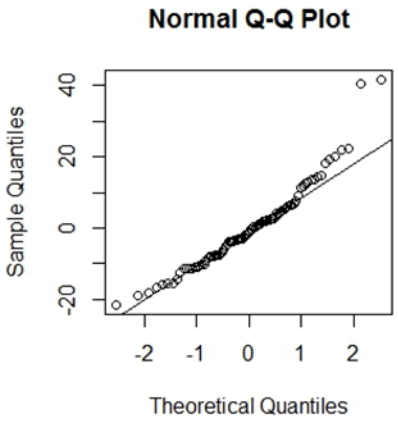




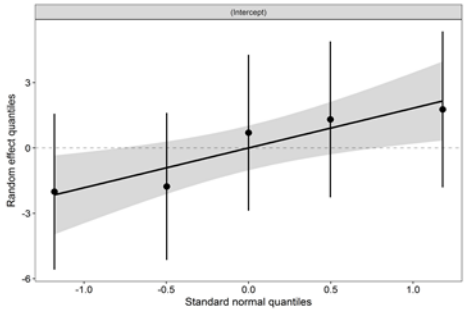
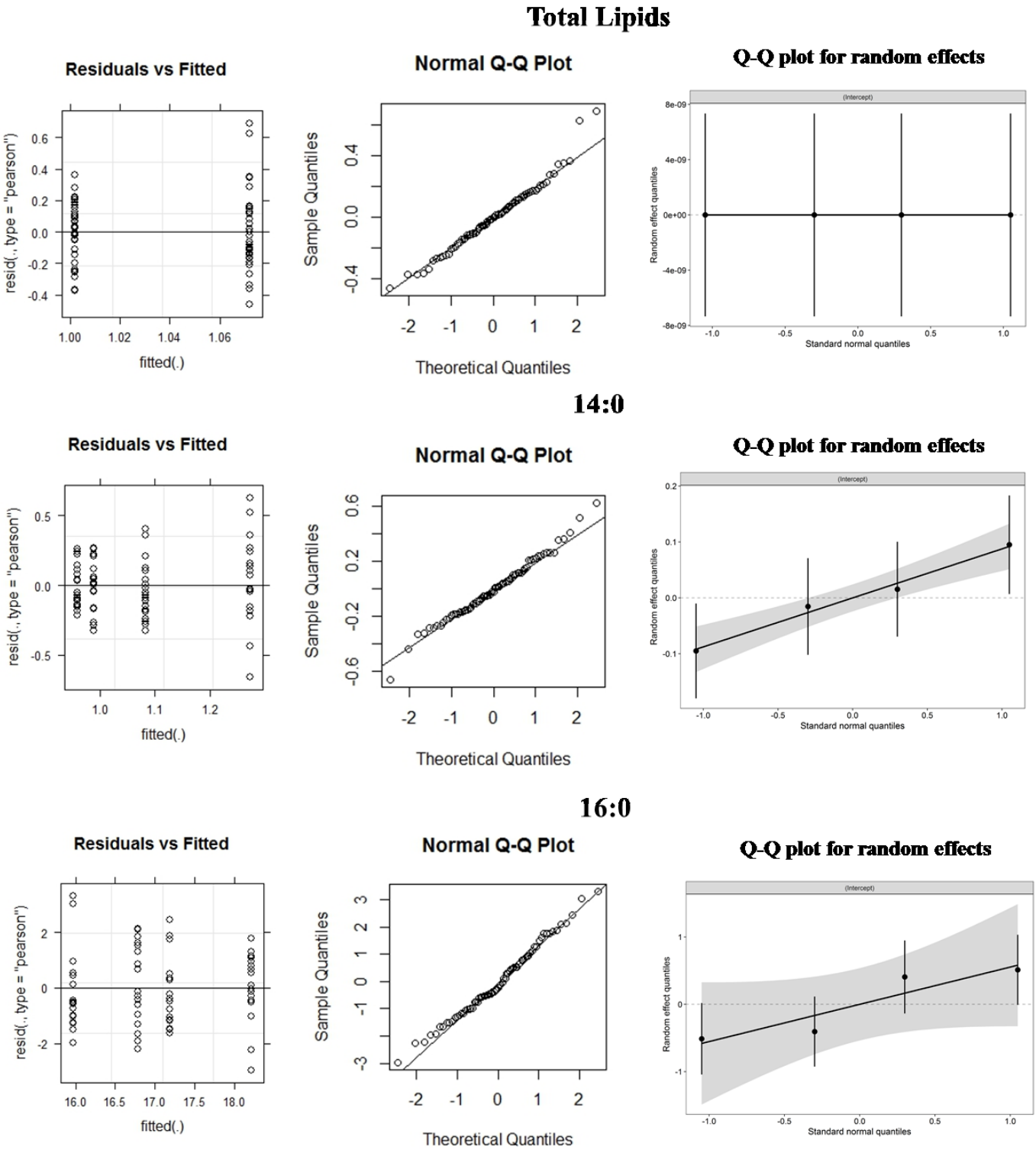


Figure C.4 Residual plots for mixed effect models for total lipid and selected fatty acids for muscle tissues of whiting sampled near and away from two fish farms and two reference sites.



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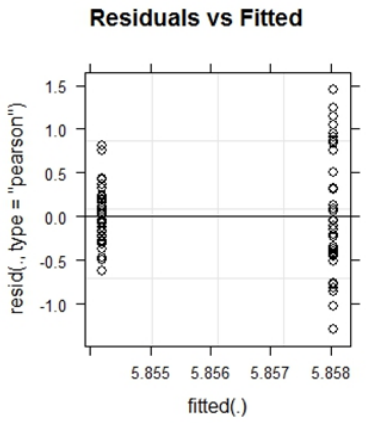

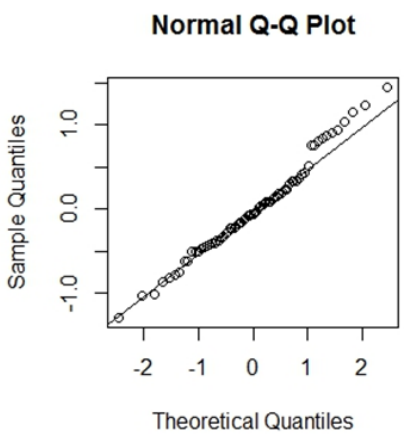

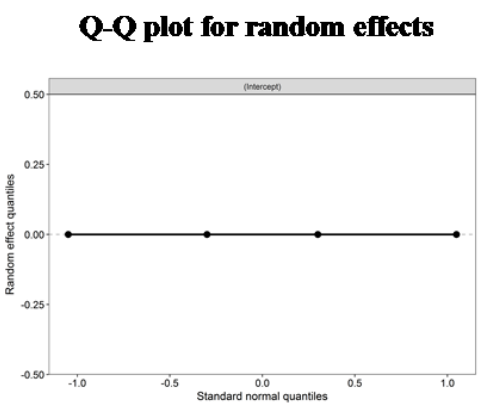

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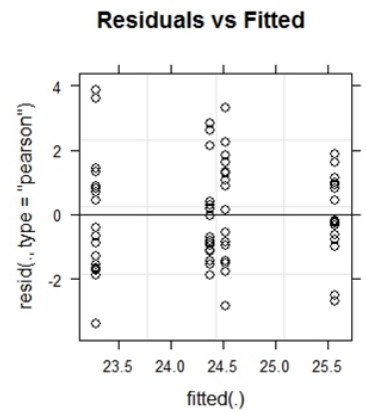

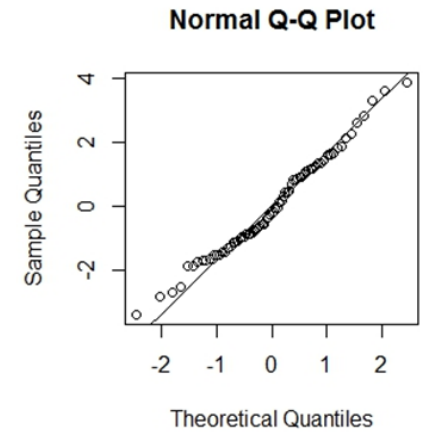

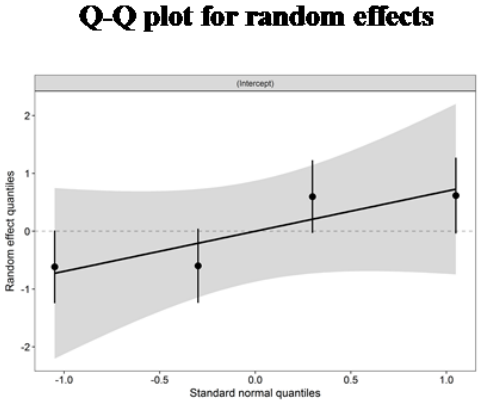

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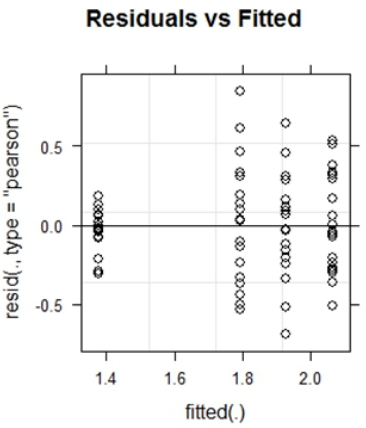

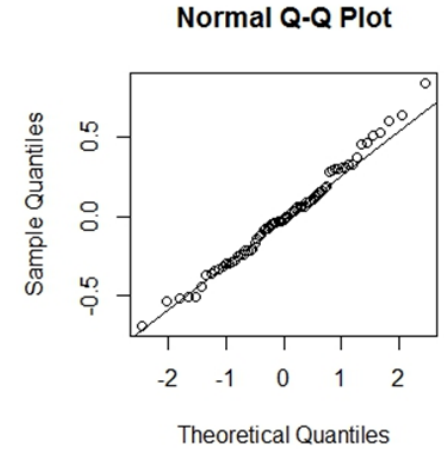

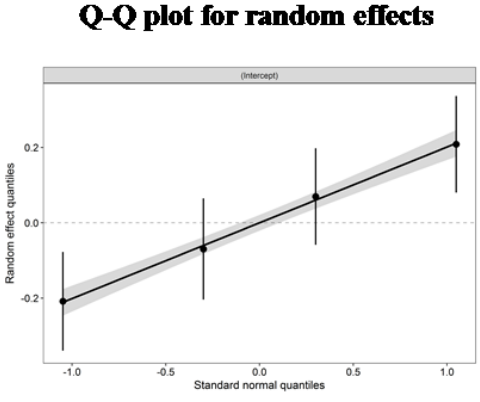

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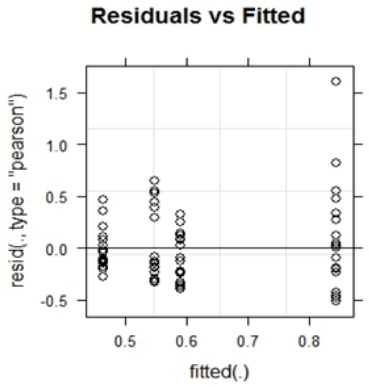

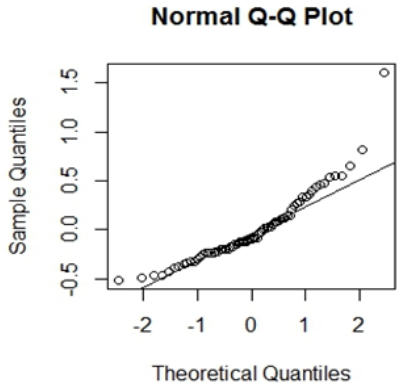

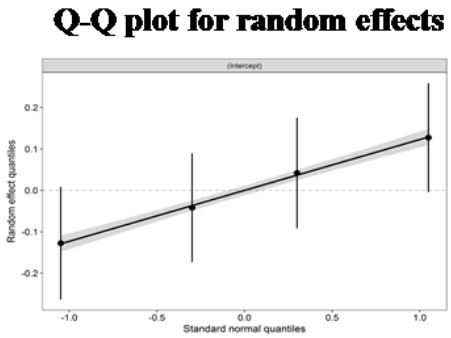

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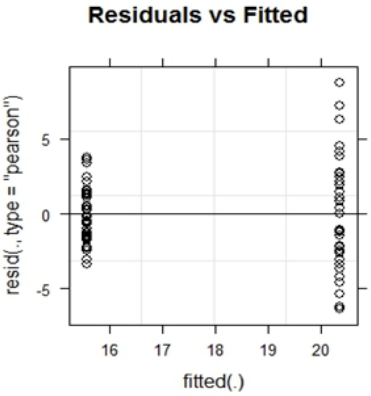

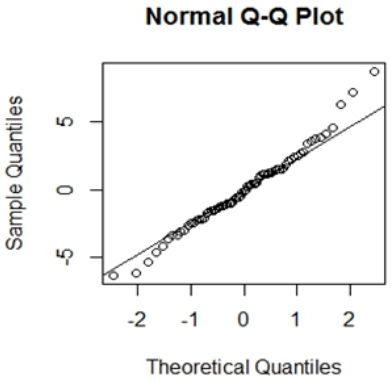

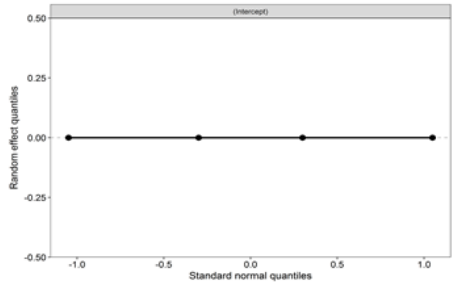

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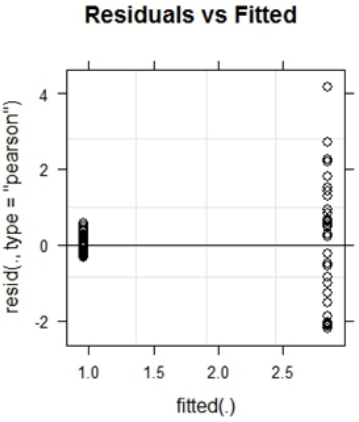

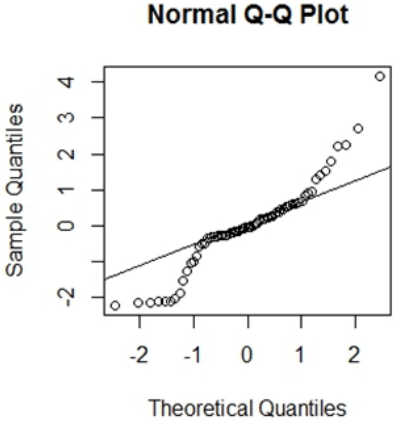

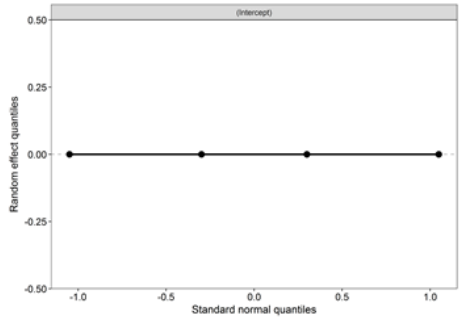

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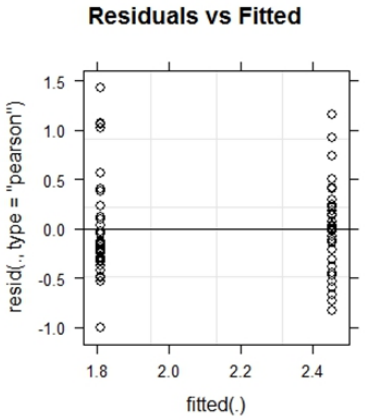
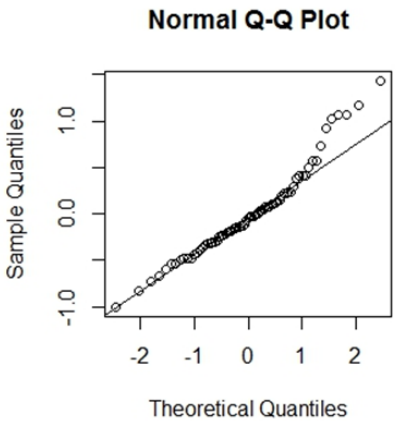
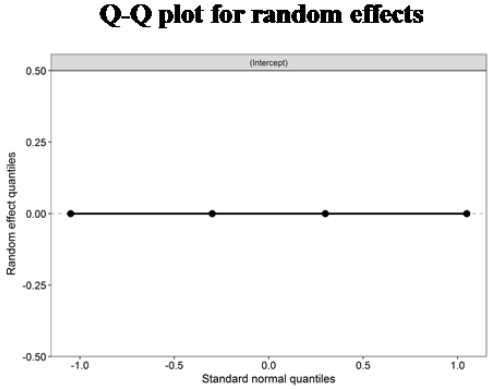
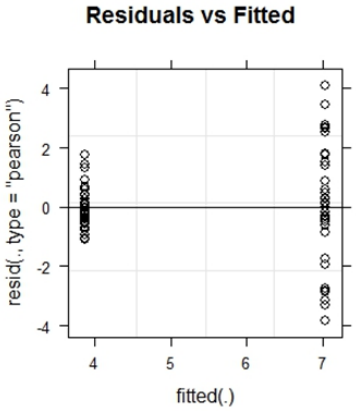
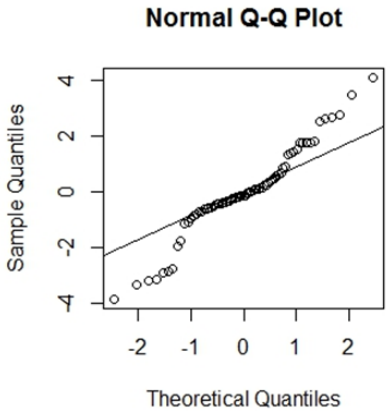


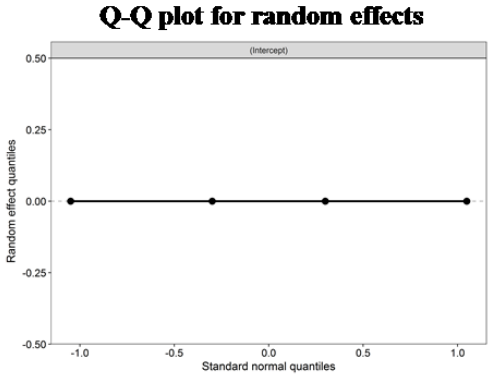




Total n-6 PUFAs







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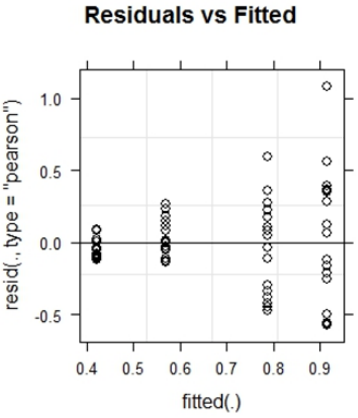

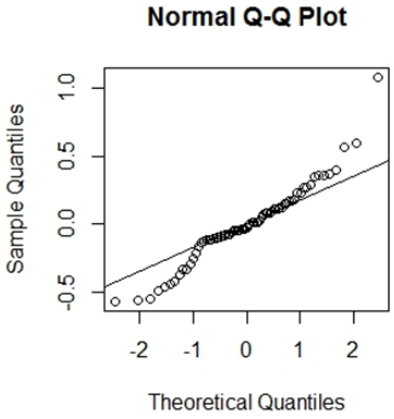

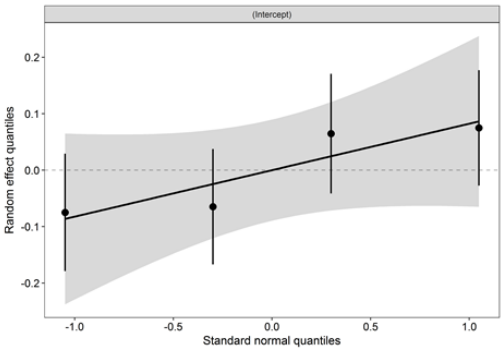

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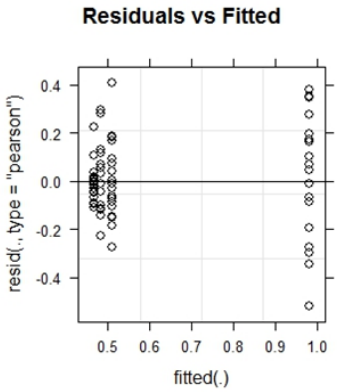

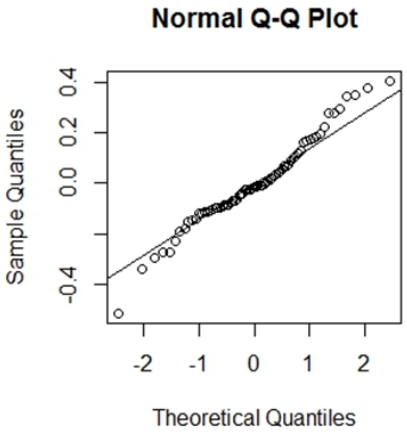

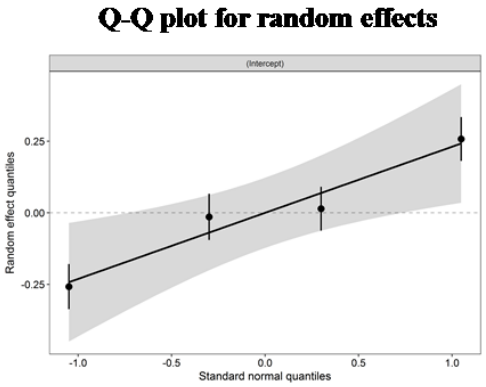

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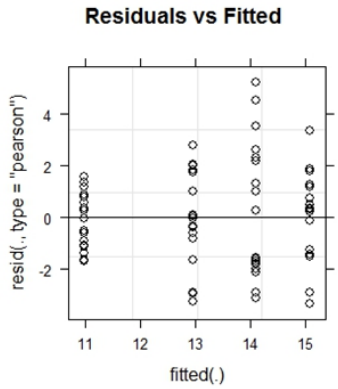

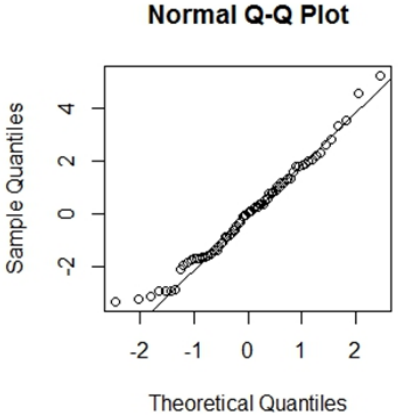

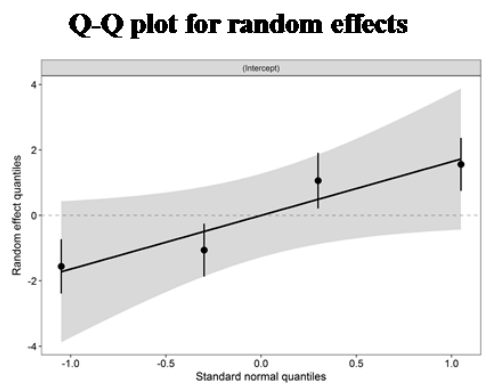

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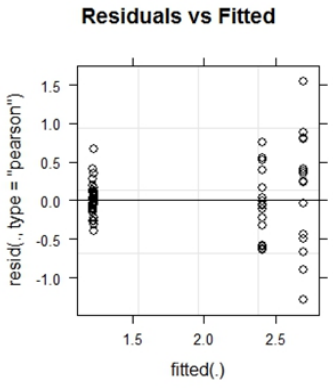

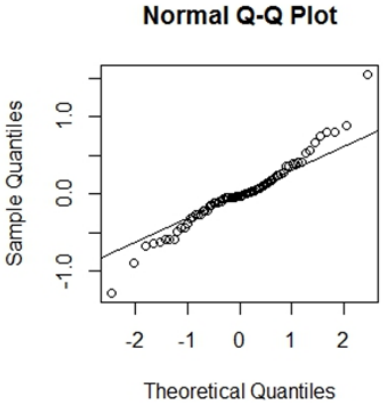

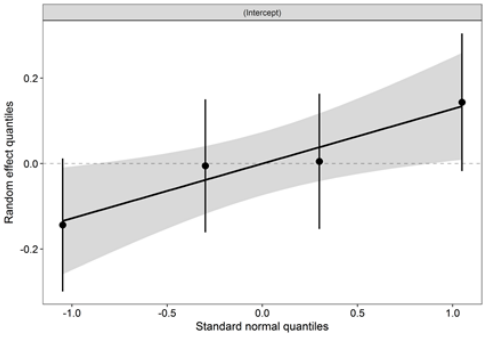

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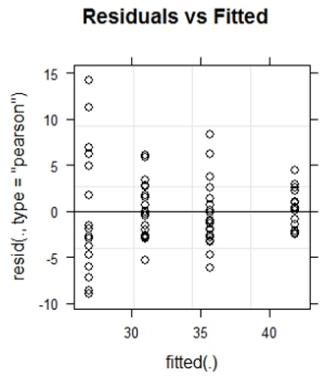

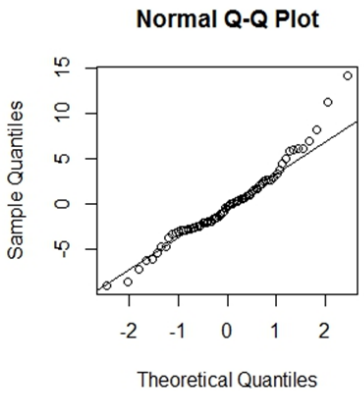

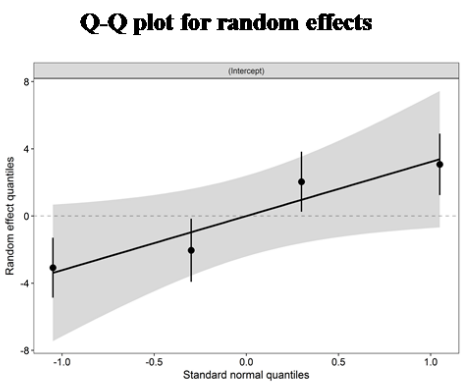

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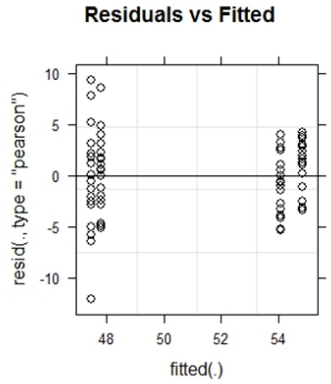
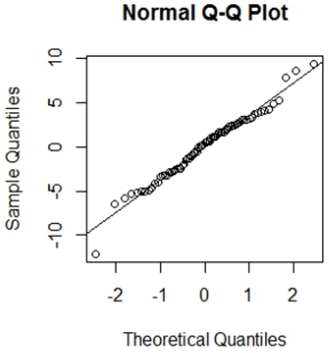
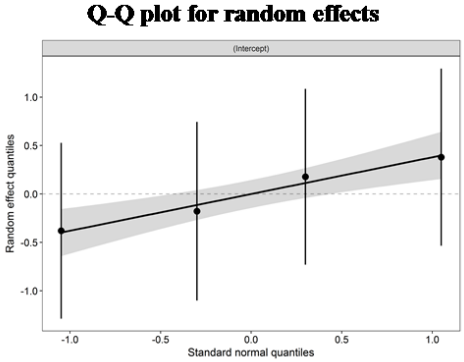
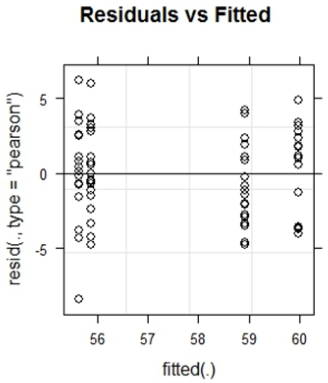
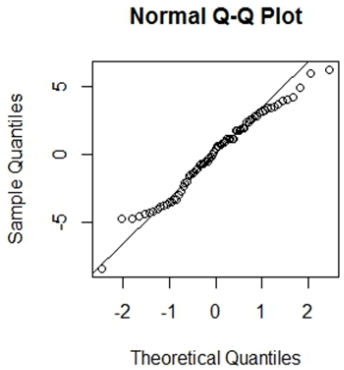


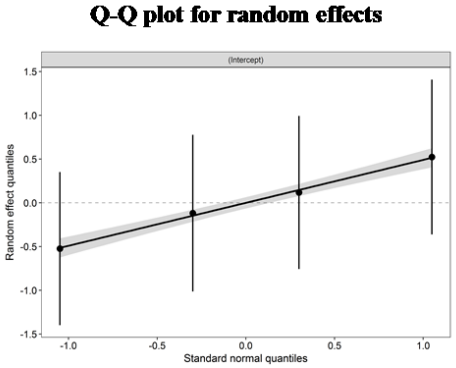




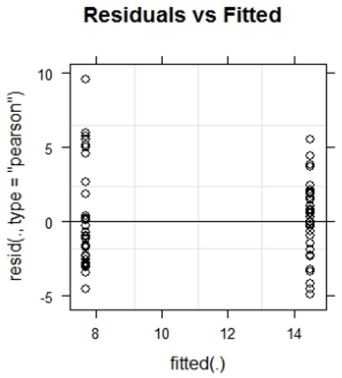
Total PUFAs

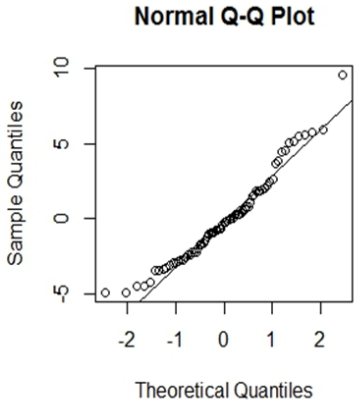


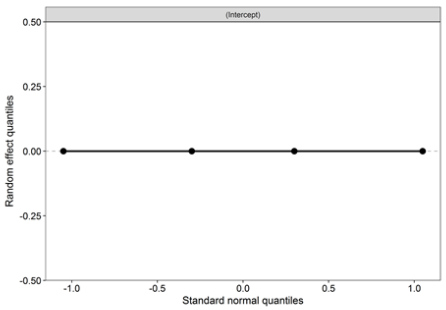




n-3/n-6







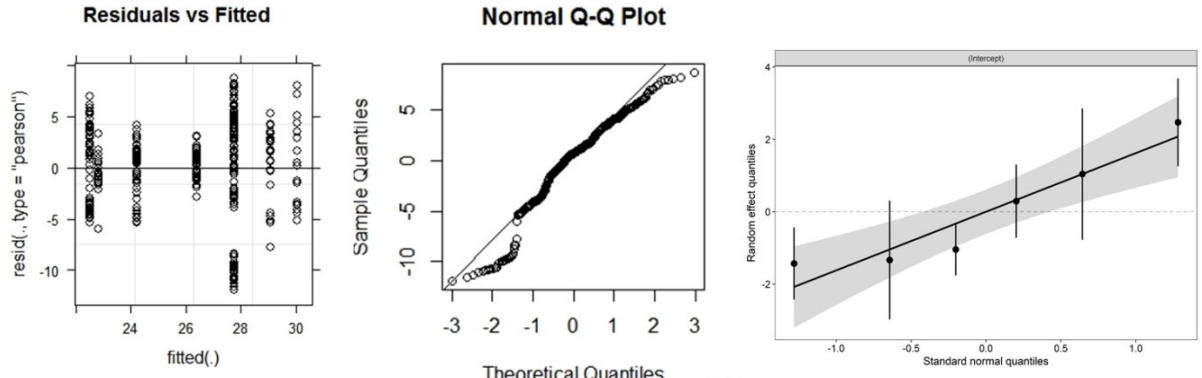
APPENDIX D

ADDITIONAL INFORMATION FOR CHAPTER 6

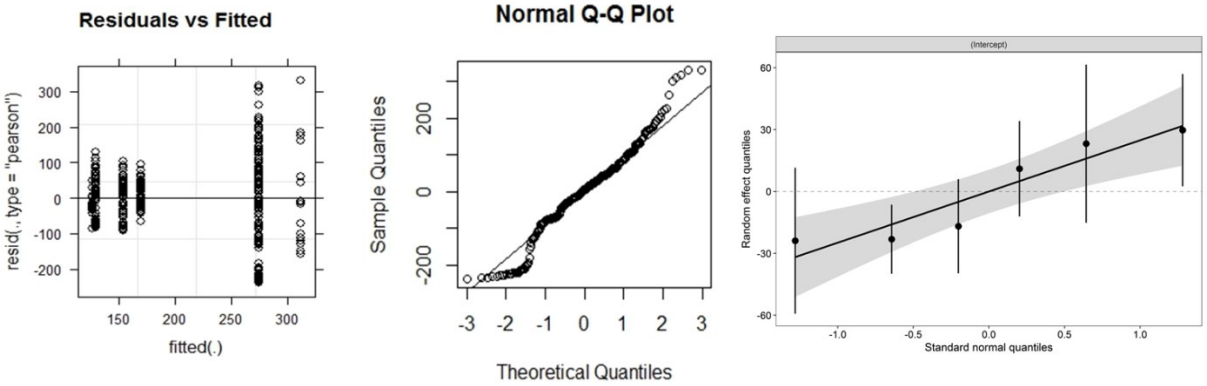
This appendix includes diagnostic plots for all statistical models used in Chapter 6.

Figure D.1 Diagnotic plots for linear mixed effect models for length (cm), mass (g), FCI and HSI for mackerel sampled near and away from sea cages.

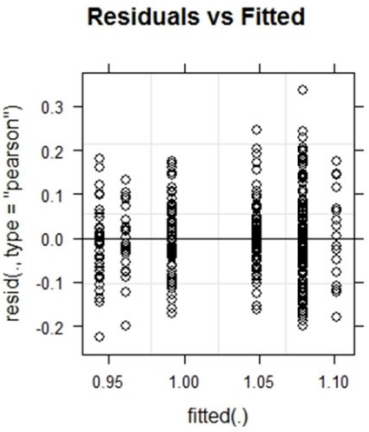
Length

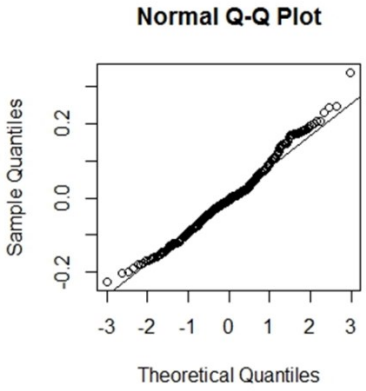


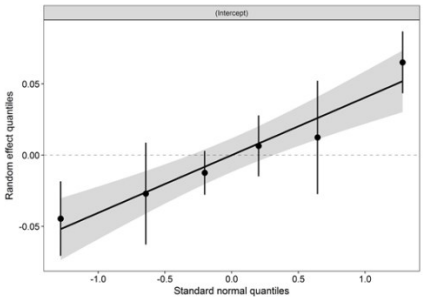
Mass



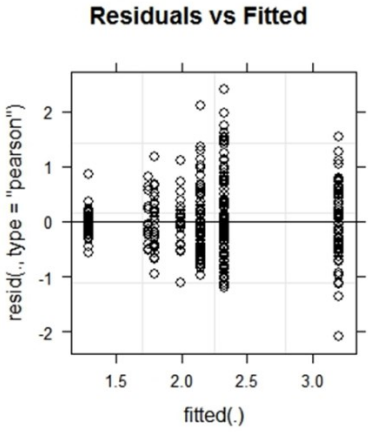
FCI

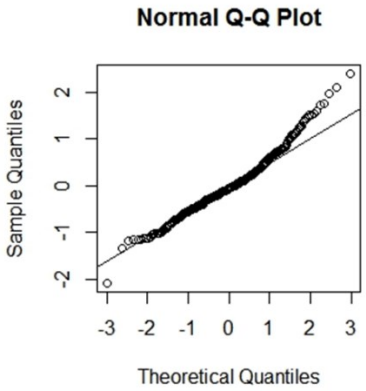

Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles


(Intercept)
Random effect quantiles
Standard normal quantiles

HSI


Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles

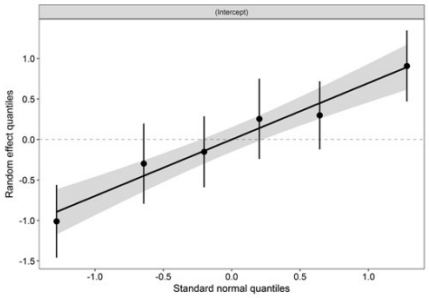
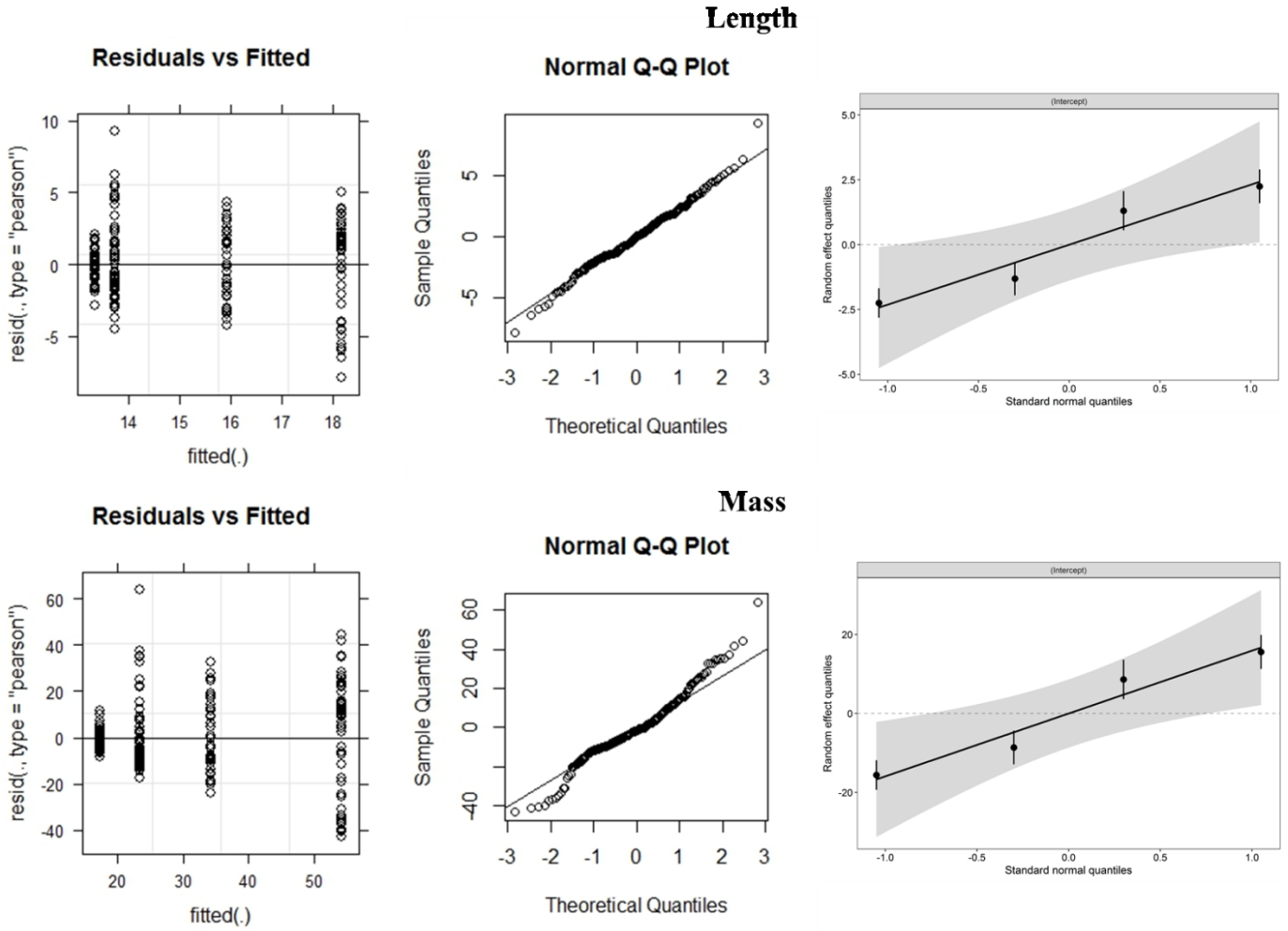
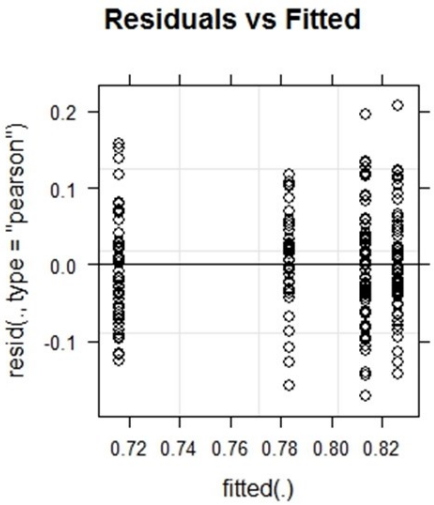
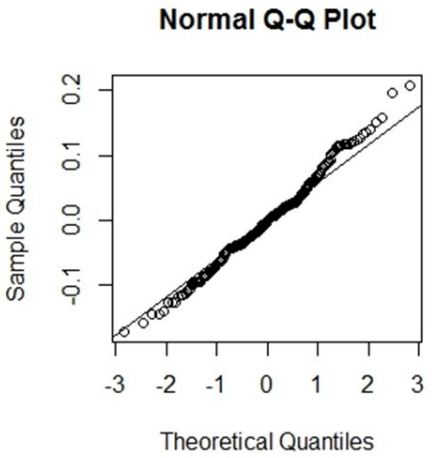

(Intercept)
Random effect quantiles
Standard normal quantiles

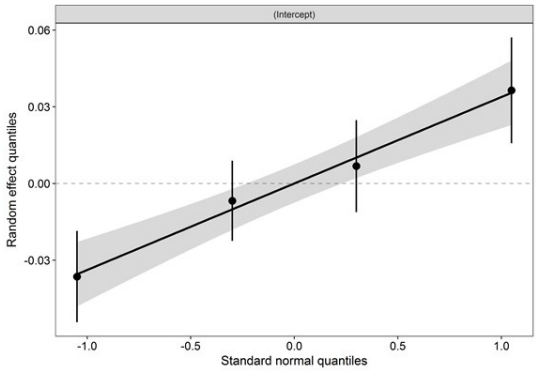
Figure D.2 Diagnotic plots for linear mixed effect models for length (cm), mass (g), FCI and HSI for whiting sampled near and away from sea cages.



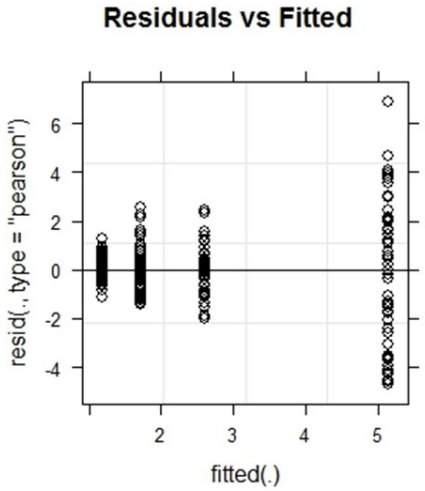
FCI

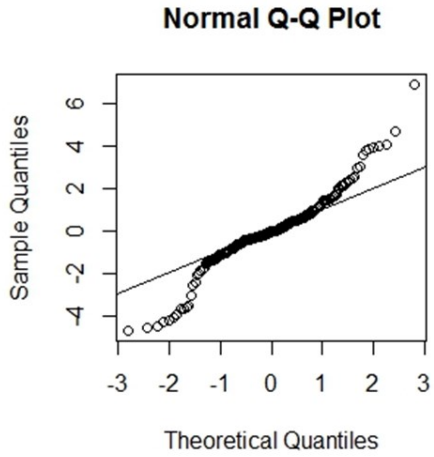

Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles


(Intercept)
Random effect quantiles
Standard normal quantiles

HSI


Residuals vs Fitted
resid(., type = "pearson")
fitted(.)


Normal Q-Q Plot
Sample Quantiles
Theoretical Quantiles

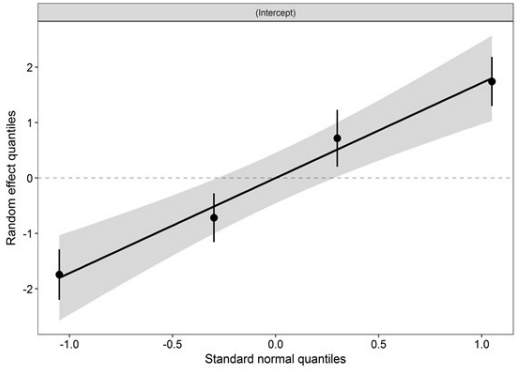

(Intercept)
Random effect quantiles
Standard normal quantiles

Figure D.3 Diagnotic plots for ANOVA/ANCOVA models for length (cm), mass (g), FCI and HSI for saithe sampled near and away from sea cages.

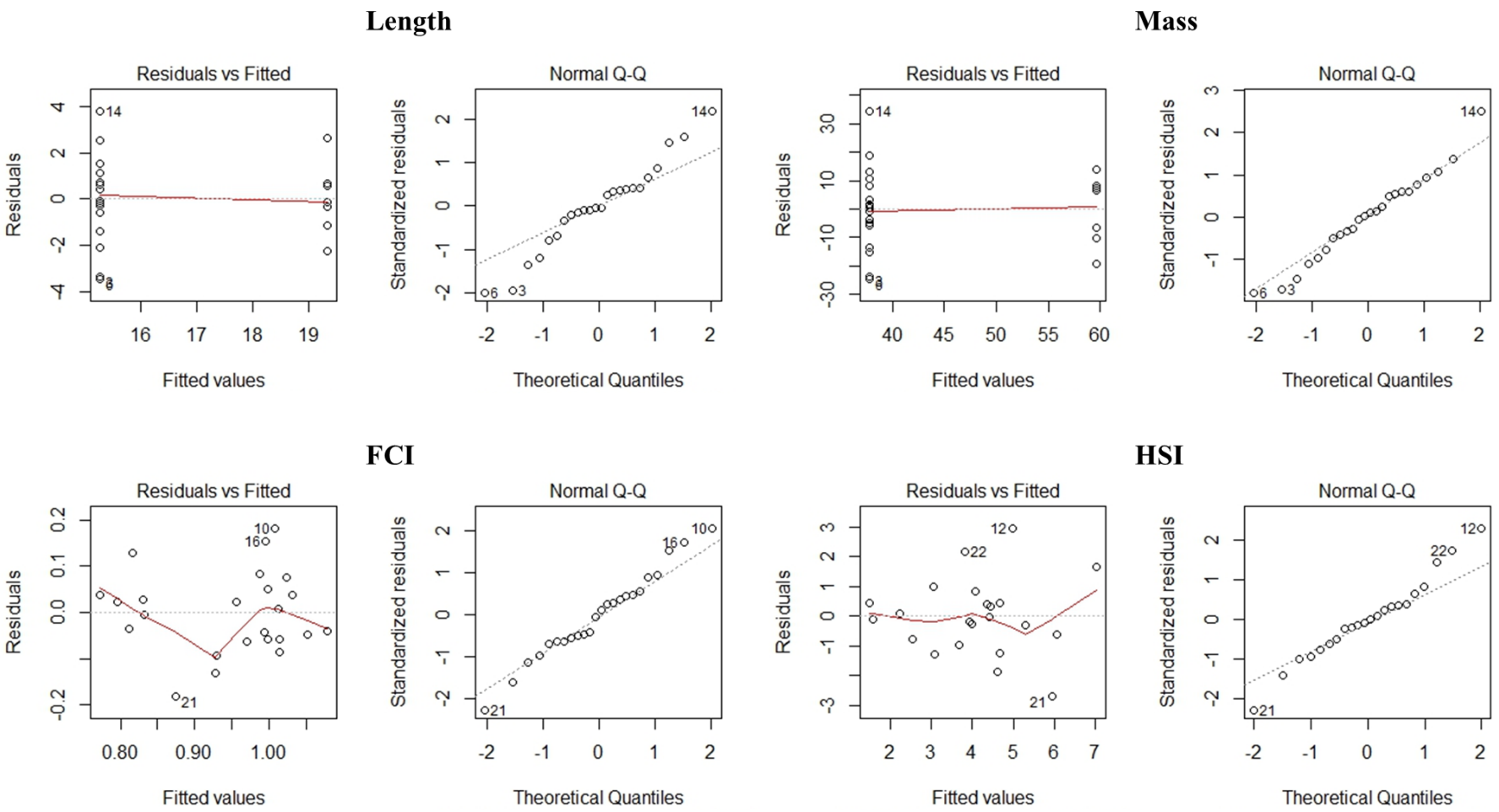
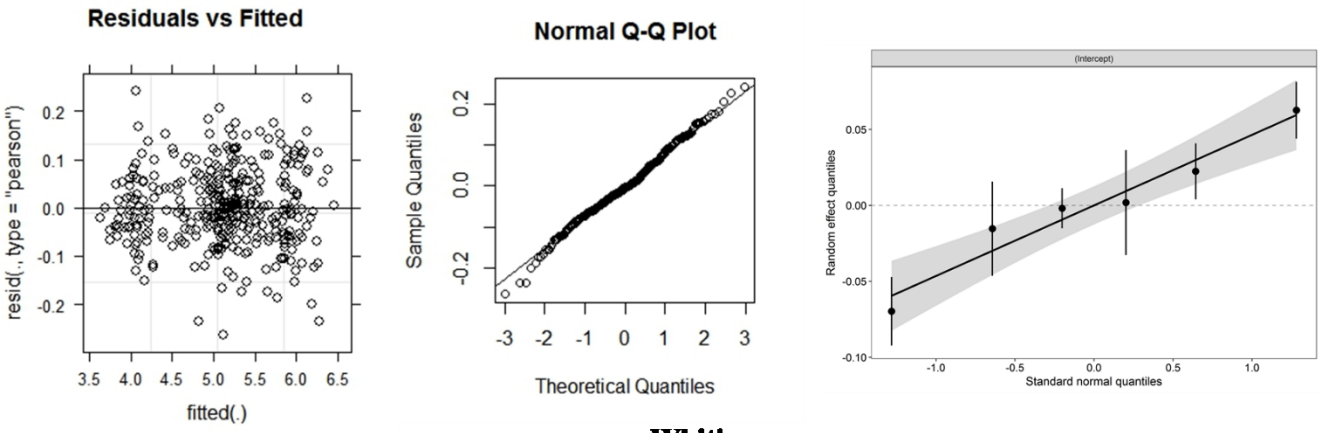
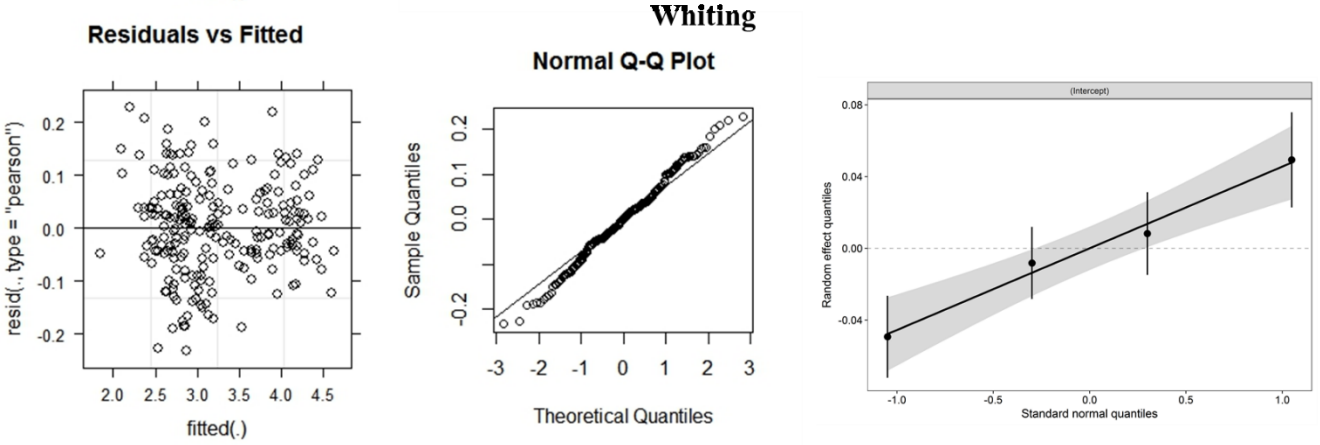


Figure D.4 Diagnotic plots for length-mass relationships for mackerel, whiting and saithe sampled near and away from sea cages.

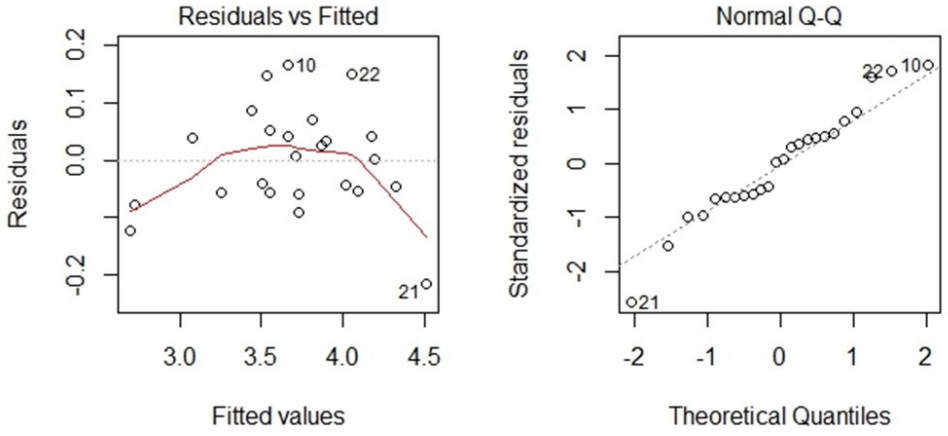
Mackerel



Whiting



Saithe



APPENDIX E
MODEL IMPLEMENTATION: CHAPTER 7

The following code was used for the construction of a phase space model for mackerel. The same code was applied for whiting except the matrix inputs were changed.

E.1 Create basic Leslie population matrix

E.1.1. Matrix inputs for mackerel

```
mackerelSurvival <- c(0.86, 0.82, 0.76, 0.76, 0.76, 0.85, 0.84, 0.72, 0.71, 0.69, 0.81, 0.71)
mackerelFertility <- c(0.11, 0.36, 0.70, 1.03, 1.37, 1.53, 2.02, 2.10, 2.33, 2.60, 3.60, 4.16)
```

E.1.2 Building the matrix

```
buildMatrix <- function(surv, fert) {
  k <- length (fert)
  A <- matrix (0, nrow=k, ncol=k) ### make k x k matrix of zeros
  A[row(A) == col(A) + 1] <- surv ### put survival on the subdiogonal
  A[1, ] <- fert
  Return (A)
}
mackerelMatrix <- buildMatrix(mackerelSurvival, mackerelFertility)
mackerelMatrix[12, 12] <- mackerelMatrix[12, 11]
print(mackerelMatrix)
## the intrinsic rate of population growth is given by the eigenvalue
## and the stable population structure is the eigenvector
getGrowthRate <- function(myMatrix){
  eig <- eigen(myMatrix, only.values=T)$values
## This returns N eigenvalues and vectors and most of these are complex numbers.
## Then, pull out one of these numbers where imaginary part is zero.
realEigs <- which(Im(eig)==0)
return (Re(eig[realEigs[1]]))
}
```

```
intrinsic <- getGrowthRate(mackerelMatrix)
```

E.2 Build positive and negative matrices

```
## To build the positive matrix use an arbitrary multiplier of 0.5 (upper assumed limit).
## Positive effect of fish farm on wild fish is 50% increase in fecundity across all ages.

## To build the negative matrix use an arbitrary multiplier of 0.5 (lower assumed limit).
## Negative effect of fish farm on wild fish is 50% increase in mortality across all ages.

## This is assuming that all age classes come around the sea cages.
```

E.2.1 Positive matrix

```
maxFertPlus <-  mackerelFertility * seq(from=0.5, to=0.5,
length.out=length(mackerelFertility))

positive <- buildMatrix(rep(0, 12), maxFertPlus)

positive[12,12] <- positive[12,11]

print (positive)
```

E.2.2 Negative matrix

```
maxSurvMinus <--mackerelSurvival *seq(from=0.5, to=0.5,
length.out=length(mackerelSurvival))

negative <- buildMatrix(maxSurvMinus, rep(0, 12))

negative[12,12] <- negative[12,11]
```

E.3 Phase space models

```
## The phase space model is the combinations of postive and negative matrices.

## The number of steps to take to build up to a maximum effect are:

buildPhaseSpace <- function(natural, positive, negative, steps){
 phaseSpace <- matrix(NA, nrow=steps, ncol=steps)
 for(i in 1:steps){
  for(j in 1:steps){
   phaseSpace[i, j] <- getGrowthRate(natural + (i/steps)*positive + (j/steps)*negative)
  }
 }
 return(phaseSpace)
```

```
}
mackerelPhaseSpace <- buildPhaseSpace(mackerelMatrix, positive, negative, 50)
maxRate <- max(mackerelPhaseSpace)
```

E.4 Plot the phase space model

```
library (ggplot2)
library (RColorBrewer)
library (reshape2)
pS <- melt (mackerelPhaseSpace)
names (pS) <- c("positive", "negative", "growthRate")
mackerel_phase <- ggplot (pS,aes(x=positive,y=negative)) +
        geom_tile(aes(fill=growthRate)) +
scale_fill_distiller (palette="RdBu", limits=c(0.5, 1.5),
                space="Lab",  direction=1,
                guide=guide_colourbar(reverse = TRUE), name="Population Growth
Rate")
        stat_contour (aes(z=growthRate), breaks=c(1), linetype=1, colour='black') +
        stat_contour (aes(z=growthRate), breaks=intrinsic, linetype=2, colour='black')
+
        scale_x_continuous (expand=c(0,0))+
        scale_y_continuous (expand=c(0,0))+
        coord_fixed () + theme_bw () +
           theme (axis.text.x=element_text(size=9, colour="black", family="Times
New Romans"),
                axis.text.y=element_text (size=9, colour="black", family="Times New
Romans"),
                axis.title.y=element_text (size=9, family="Times New Romans"),
                axis.title.x=element_text (size=9, family="Times New Romans"),
                plot.title = element_text (size =9, family="Times New Romans"),
                panel.border=element_rect (colour="black"),
                legend.title=element_text (size=10, family="Times New Romans")) +
          ggtitle ("Phase Space Model for Mackerel") +
```

```
        xlab ("Positive: % Maximum Arbitrary Fecundity Increase") +
        ylab ("Negative: % Maximum Arbitrary Mortality Increase") +
        labs ("Population Growth Rate")
print (mackerel_phase)
library (cowplot)
save_plot ("Mackerel_BASIC_PHASE.png", mackerel_phase, base_aspect_ratio=1.8)
```

E.5 Stable age distribution and elasticity analysis

```
#install.packages ("popbio")
library (popbio)
lambda (mackerelMatrix)
stable.stage (mackerelMatrix)
eigen.analysis (mackerelMatrix)
table_mackerel <- elasticity (mackerelMatrix)
print (table_mackerel)
```

APPENDIX F
PARAMETRISATION OF FUNCTIONAL GROUPS IN ECOPATH MODELS: CHAPTER 8

This appendix provides supplementary information for the 14 functional groups used in the Ecopath models built in Chapter 8. The functional groups included: seabirds, mackerel, other fishes, juvenile whiting, crustaceans, echinoderms, zooplankton, polychaetes, farmed fish, farmed mussels, seaweed, phytoplankton, artificial feed, and detritus.

F.1 Seabirds

Seabirds in the UK are monitored by the Joint Nature Conservancy Committee (JNCC) and the Royal Society for the Protection of Birds (RSPB). Seabirds were noted near the fish farm in Loch Melfort during fieldwork in 2013 and 2014. Individual cormorants were noted during the sampling for mackerel and whiting near the sea cages in two out of nine trips in 2014. No seabird surveys were undertaken during the fieldwork. Seabirds have been reported during a sanitary survey of Loch Melfort conducted in 2015 (Scottish Sanitary Survey Report 2015). Amongst breeding colonies of common terns (*Sterna hirundo*), Arctic terns (*Sterna paradisaea*), common gull (*Larus canus*) and black headed gull (*Chroicocephalus ridibundus*), other seabirds have been noted including oystercacthers, cormorants, heron and eider ducks (Scottish Sanitary Survey Report 2015).

Biomass, production and consumption

Data for seabirds in Loch Melfort was extracted from the Seabird Monitoring Programme Database[21]. Seabird counts were extracted only for Eilean Coltair and Sgeir na Caillich found within Loch Melfort. The datasets included seabird counts for Scotland from 1986 to the latest update which was 2010. For the models, I used data available for the year 2009. The main species were black-headed gull, common gull, arctic tern and common tern. Other birds, mainly gulls, cormorants and heron were also included. The counts given in Table F8.1 are of breeding pairs and do not include the proportion of non-

[21] www.jncc.gov.uk/page-4460 [Accessed: 04 February 2018].

breeding birds. The proportion of non-breeders was assumed to be 20% of the breeding pairs (Furness 1990) and were added to the total counts. The biomass of each species was calculated by multiplying the number of birds by their body mass and the total time assumed they spend in the area. The mass of each species was obtained from Tasker and Furness (1996). The time each species spent in the area was obtained from Furness (1994) for arctic and common terns and from Tasker and Furness (1996) for black-headed and common gulls (Table F8.1).

The survival rates for black-headed gull, common gull, common tern and arctic tern used to calculate the mortality rates for the model were 0.825, 0.80, 0.860, 0.875, respectively (Furness and Wade 2012). The mortality rates were calculated using $S = e^{-Z}$ where S is survival rate and Z is mortality rate. As there was lack of data on the extra birds that were included in the group, the final P/B was obtained from the model by Bailey et al. (2011). The production rate for the seabird group was set at P/B=0.4 which was taken from Bailey et al. (2011) (Table F8.1).

The consumption rate (daily rate of fish consumed in g) was estimated from Nilsson and Nilsson (1976) using the following equation:

$$\log DR = -0.293 + 0.85 \times logW \qquad (eq.F.1)$$

where DR is the daily ration in g and W is the mean body mass of the species (g). Although the equation is for daily food consumption in piscivorous birds in freshwater environments it is often used to approximate the daily food consumption in marine seabirds (see Heymans et al. 2016). After obtaining the daily consumption for each species the Q/B values were obtained by dividing the daily ration by the mass of the seabird and then multiplying by 365. The final Q/B for the seabirds was weighed on the total biomass of the group. However, as the Q/B value for the additional birds was not known the quantity was estimated by the model using a P/Q valued of 0.015 (Haggan and Pitcher 2005) (Table F8.2).

Diet

Most seabirds consume small pelagic fishes, young gadoids, crustaceans and cephalopods (Hunt et al. 1996; Mitchell et al. 2004). The diet of the group was based on previous Ecopath models for the West coast of Scotland (Haggan and Pitcher 2005) and

Alexander et al. (2015) and diets from the literature. The diet of black-headed and common gulls in aquatic habitats and shores includes fish, polychaete worms, cockles, crabs amongst other crustaceans (Vernon 1972; Tasker and Furness 1996; Kubetzki et al. 1999). Diet of common and arctic tern consists mainly of fish and to a lesser extent crustaceans (Eglington and Perrow 2014 and references therein). The final diet of the group was constructed by averaging different components and adjusting for the models. The final diet of the seabird group was a combined diet consisting mainly of fish and crustaceans, molluscs, echinoderms, and polychaete worms. For scenarios 1-3 the diet incorporated farmed fish and mussels. The farmed fish and farmed mussels were incorporated at 1% in the diet of the seabirds. This is assuming a minimum influence in the diet.

Table F8.1 Parameters, B, P/B, Q/B, for seabirds used in the model.

Species	Common name	Count (in pairs)	Method	Weight (g)	Days spent in area	Biomass (t/km^2)	P/B	Q/B
Chroicocephalus ridibundus	Black-headed gull	10	Occupied nests	250	180	0.0003	0.19	8.362
Larus canus	Common gull	28	Occupied nests	380	180	0.0012	0.22	6.421
Sterna paradisaea	Arctic tern	19	Occupied nests	100	200	0.0002	0.13	14.905
Sterna hirundo	Common tern	234	Occupied nests	125	100	0.0019	0.15	12.948
Other birds	Gulls, cormorants, heron	-	-	800-2200	180-365	0.0064	-	-
Final	-	-	-	-	-	0.01	0.4	-

F.2 Mackerel

During fieldwork of 2013 and 2014, I sampled mackerel near the fish farm in Loch Melfort. On some visits to the fish farm there were schools of (~30-100 individuals) mackerel chasing after clupeids and on others only few mackerel were noted (see Appendix A).

Biomass, production and consumption

Biomass was estimated from the model using an Ecotrophic Efficiency of 0.95. The production to biomass ratio was calculated using the assumption that P/B = Z (Allen 1971) and Z = natural + fishing mortality where Z = total mortality. The natural mortality used was 0.39 obtained from FishBase and approximate fishing mortality of 0.25 was used (ICES 2016). The final P/B value used for the model was 0.69/year. The P/B value was similar to the value obtained in the model by Alexander et al. (2015). The consumption to biomass ratio used for the model was 4.4/year and was obtained from FishBase[22].

Diet

The diet of mackerel in the Northeast Atlantic is dominated (> 50%) by zooplankton (Pinnegar 2014; Bachiller et al. 2016). The diet for mackerel was initially estimated from Pinnegar et al. (2015) using data for the latest three years available and Langøy et al. (2006). Based on diet from mackerel caught in the loch in 2013 and 2014 diet was dominated by fish (see Chapters 4 and 5). Therefore, the final diet was adjusted to reflect fish as the main component of the diet of mackerel in the loch system. For scenarios 1 and 2, 15% of the diet in mackerel was assumed to contain artificial feed. The number is based on the stomach content data from Chapters 4 and 5.

Recreational fishing

There is no commercial fishing for mackerel in Loch Melfort but there is some recreational fishing. Some mackerel are also caught for bait for the lobster fishery (anecdotal accounts from fisherman in the Loch). If on average 30 fisherman catch 15

[22] http://www.fishbase.org/search.php [Accessed: 04 February 2018].

mackerel per day (guesstimate) (each weighing ~ 500 g) during the summer season (~ 4 months) then the final catches were estimated to be 0.0122 tonnes/km^2.

F.3 Other fishes

The group included juvenile gadoids, flatfishes and wrasse (Family Labridae) which is similar to the group used for inshore fish in the model by Haggan and Pitcher (2005). During the fieldwork to Loch Melfort, gadoids, flatfishes and wrasse were observed (see Appendix A) and therefore this group was assumed to be similar to the one by Haggan and Pitcher (2005).

Biomass, production and consumption

There is a lack of information for this group and therefore the biomass was estimated using an EE of 0.95. A P/B of 5/year was used for the model (Haggan and Pitcher 2005). The same value was used in this model. To estimate the Q/B value a P/Q value of 0.3 was used (Christensen and Pauly 1992).

Diet

The diet for this group was a modified diet based on the inshore fish group from the model of Haggan and Pitcher (2005).

F.4 Juvenile whiting

Juvenile whiting are widely distributed in coastal inshore areas on the West Coast of Scotland from June to December and move offshore around 1 year of age (Bailey et al. 2011). During both years of fieldwork in Loch Melfort whiting were sampled near the sea cages in higher numbers than other gadoids such as saithe and cod. Thus, it was included as a separate group in the model scenarios.

Biomass, production and consumption

The biomass of whiting was estimated by the Ecopath model using an Ecotrophic Efficiency of 0.95. As no data was available to estimate the P/B of whiting for the sea loch a P/B value of 1.7/year was used for the model which was based on the model by Alexander et al. (2015) for the group immature whiting. The consumption to biomass

value was obtained using the lifehistory tool in FishBase where an average length of 12.2 cm (see Chapter 5) was entered and a Q/B value of 7.0/year was calculated.

Diet

The diet for juvenile whiting was based on the diet presented in the Ecopath model by Alexander et al. (2015). The diet also incorporated the results of the stomach content analysis for whiting caught in 2013 and 2014 in the loch system (see Chapter 5 and 6). The diet was mainly composed of benthic invertebrates. For scenarios 1 and 2, the diet included artificial feed at an assumed proportion of 0.30. This number was based on the stomach content analysis in Chapter 5 (see also Chapter 6) for whiting sampled near the sea cages in Loch Melfort. Based on the FA analysis the proportion of artificial feed in the diet might be higher; however a minimal proportion was included in the model scenarios.

F.5 Crustaceans

This group included crabs, lobsters and nephrops. Lobster (*Homarus gammarus*) (Appendix A) and crab (*Cancer pagurus*) (Appendix A) were caught by fish farm staff near the sea cages (see Appendix A).

Biomass, production and consumption

The biomass for the crustaceans was estimated by the model with an Ecotrophic Efficiency of 0.95. The P/B value was based on the previous model of the West Coast by Haggan and Pitcher (2005). The P/B value was an average of the nephrops and crabs/lobsters groups. The value was estimated at 3.75/year. However, the biomass appeared too high and the P/B value was reduced to 2/year. The consumption Q/B was estimated by the model using a P/Q value of 0.15 (Christensen 1995).

Diet

The diet was based on the previous models by Haggan and Pitcher (2005) and Alexander et al. (2015). The diet was averaged to consist mainly of detritus, seaweed, zooplankton, echinoderms, molluscs, and polychaetes (see Haggan and Pitcher 2005). The diet also incorporated consumption of particulate waste (e.g. faeces) from both fish

and mussel farms at a minimum inclusion of 1%. The number was assumed to be a minimal farming influence on the diet.

Fisheries

There is no industrial level fishing, within the loch, for crustaceans but there is a small-scale fishing using creel pots. Using a very rough estimate the crustacean (mainly lobsters and crabs) fishery was calculated using catch per unit effort (CPUE) (total weight landed (kg)/number of creels used) for lobsters 0.2, and CPUE for brown crab of 0.542 (Coleman 2014). If on average 30 creels are used within the loch every month the total lobster and crab fishery was estimated at 0.0220 tonnes/km^2. The number of creels were based on the approximate number of creels noted on one of the piers in Loch Melfort.

F.6 Echinoderms

The echinoderms caught during the fieldwork of 2013 were common sea urchin (Appendix A), common sea star (*Asterias rubens*) (Appendix A) and brittle stars (see Appendix A; Table A.1; Figure A.9). These are assumed to be some of the common echinoderms in the loch.

Biomass, production and consumption

The biomass for the echinoderms was estimated by the model with an EE of 0.95. The P/B value for the group was taken as the average of the P/B values for brittle stars, sea urchins and starfish reported in the model for the North Sea by Mackinson and Daskalov (2007) and the P/B of 4/year used for the West Coast of Scotland model (Haggan and Pitcher 2005). The final P/B value for the group was approximated at 2.135/year. The value falls within the general range (0.5-2.5) of P/B values for echinoderms (Redant 1989). The consumption value (Q/B) was unknown and therefore a P/Q of 0.15 was used (Mackinson and Daskalov 2007) to estimate the Q/B value by the model.

Diet

The diet composition was obtained as a combination from Haggan and Pitcher (2005) and Stanford and Pitcher (2004). The diet mainly consisted of molluscs,

polychaetes, seaweed and phytoplankton. Artifical feed and detritus from aquaculture activities was incorporated at 1% into the diet, assuming a minimal influence.

F.7 Polychaetes

Polychaetes were the predominant benthic organisms caught during the macrobenthic sampling in 2013 at Loch Melfort (see Appendix A).

Biomass, production and consumption

The biomass of the polychaete group was estimated by the model with an EE of 0.95. A P/B value of 5/year was used for the model (Haggan and Pitcher 2005). The Q/B ratio was estimated by the model using a P/Q value of 0.3 (Haggan and Pitcher 2005). Using a P/B value of 5/year gave an overall low biomass. The P/B value was calculated by taking the mean of 1.51 used in a model for a fjordic system (Pedersen et al. 2016) and 0.9 for the North Sea (Mackinson and Daskalov 2007). The final P/B value used for the model was 1.20/year.

Diet

The diet of the group consisted of phytoplankton and detritus and was based on the model by Haggan and Pitcher (2005). Waste from both aquaculture activities was incorporated in the diet at 1%, assuming a minimal aquaculture impact.

F.8 Zooplankton

Assuming that the zooplankton in the loch immigrate from the ocean (Ross et al. 1993), the group was based on the model by Haggan and Pitcher (2005) and consisted of large and small zooplankton. In this model, both groups were joined under the zooplankton group.

Biomass, production and consumption

The biomass for the zooplankton was estimated by the model with an Ecotrophic Efficiency of 0.95. The P/B was calculated at 14/year based on the average of small (18/year) and large (10/year) zooplankton in the model of Haggan and Pitcher (2005). The Q/B value was estimated by the model using a P/Q value of 0.30 (Christensen and Pauly 1992).

Diet

The diet was a combination of the different groups in the model of the West Coast by Haggan and Pitcher (2005). A combined diet of large and small zooplankton was used. The main food items for this groups were phytoplankton followed by detritus (Haggan and Pitcher 2005).

F.9 Farmed fish

Farmed fish in Loch Melfort include sea grown rainbow trout and Atlantic halibut (see Chapter 3). Data on monthly biomass, feed, and mortalities was obtained from Scotland's aquaculture website[23].

Biomass, production and consumption

The biomass was calculated for each species and each year (2013 and 2014). The biomass for the group for both years and both farms was estimated at 19.25 tonnes/km^2 and harvested fish at 27.38 tonnes/km^2. The P/B ratio for both species was estimated at 1.45/year using an FCR of 1.3 (Gillibrand et al. 2002). The Q/B value was estimated at 1.83/year.

Diet and predators

The diet of the farmed fish was assumed to be composed of artificial feed. For scenarios 1 and 2, I assumed that mainly seabirds could feed on the farmed fish.

F.10 Farmed mussels

Blue mussels or also known as common mussels (*Mytilus edulis*) are also produced in Loch Melfort on long lines. Other shellfish are present but production is assumed negligible (Scottish Sanitary Survey Report 2015).

Biomass, production and consumption

There was no information available on the biomass and amount of harvested farmed mussels. In 2014, the mussel farm consisted of 13 lines of approximately 300 m long with 10 m droppers (Scottish Sanitary Survey Report 2015). A standing biomass of 50 tonnes

[23] Scotland's Aquaculture: http://aquaculture.scotland.gov.uk/ [Accessed: 4 February 2018].

was assumed as not all ropes appeared to be in use during an earlier visit to the loch area in 2013. This was approximated using the Farm Aquaculture Resource Management (FARM) model[24]. The total biomass in the loch was estimated at 4.95 tonnes/km^2. The values for P/B and Q/B were 2.00 and 20.000/year based on the model by Leloup et al. (2008). The P/B value falls close to the range (1.85- 2.20) for mussels in two Scottish Lochs on the West Coast (see Stirling and Okumuş 1995). The harvested biomass was estimated at 2.48 tonnes/km^2 using the P/B value of 2/year.

Diet

The diet was assumed to be mainly composed of phytoplankton (Haggan and Pitcher 2005) and detritus.

F.11 Seaweed

Seaweed is found along the coastline of Loch Melfort.

Biomass and production

The biomass of the seaweed groups was estimated using an EE of 0.5 assuming not everything is utilised in the system (Heymans et al. 2016). The P/B value was initially set at 15/year based on the West coast of Scotland model (Alexander et al. 2015). However, the biomass was too low for the loch and the P/B value was set to 5/year which was slightly lower than the average of P/B of 15/year for the West of Scotland model and a P/B of 0.49 in a similar fjordic system in Norway (Pedersen et al. 2016).

F.12 Phytoplankton

Phytoplankton biomass varies with season. Tett and Wallis (1978) noted that the phytoplankton biomass in Loch Creran increases in spring and summer and decreases in winter months which is assumed to be the case for Loch Melfort.

Biomass and consumption

A very rough estimate of the biomass of phytoplankton in Loch Melfort was based on information for Loch Etive (Wood et al. 1973). The annual phytoplankton productivity

[24] http://www.farmscale.org/ [Accessed: 4 February 2018].

for Loch Etive was reported at 70 g C/m^2 (Wood et al. 1973). Using conversion factor of 0.1 g C = 0.2 g dry weight = 1 g wet weight (Matthews and Heimdal 1980 cited in Mackinson and Daskalov 2007) the phytoplankton productivity was estimated at 700 tonnes/km^2.

A very rough estimate of 1 g C/m^2 was used as phytoplankton standing crop (Wood et al. 1973). The biomass was estimated at 10 tonnes/km^2 using the conversion factor as described previously. The P/B value was estimate at 70/year which is similar to that reported for the model of the West coast by Haggan and Pitcher (2005).

F.13 Artificial feed

The artificial feed group was considered as a second detritus group. This group was only entered for scenarios 1 and 2 when fish farming was present. The artificial feed fed to farmed fish was estimated by averaging the feed input for both species and both years (2013 and 2014). The total biomass of the feed going into the system was calculated at 35.59 tonnes/km^2. Data on the monthly feed input was obtained from Scotland's aquaculture website [25].

F.14 Detritus

Detritus has several sources in sea lochs; all the sinking dead organic material including phytoplankton and faecal pellets, macroalgae that decompose, terrestrially derived detritus, and material that resuspends (by wind and tides) from the bottom sediment in the water column (Ansell 1974). Overnell and Young (1995) noted that about 80% of the sediment in Loch Linnhe is resuspended material. In a study on the organic carbon budget in Loch Creran it was noted that the organic material from river discharge and phytoplankton are major contributors to the organic input in the loch (Loh et al. 2010). There are no studies to my knowledge on the organic carbon budget in Loch Melfort. Organic input into the loch can possibly come from sewage discharges, agriculture in surrounding area (mainly cattle and sheep), there are a number of streams and the River Oude flowing into the loch (Scottish Sanitary Survey Report 2015). Additionally, there are fish and mussel farms that contribute to the overall organic load in the loch.

[25] Scotland's aquaculture website: http://aquaculture.scotland.gov.uk/ [Accessed: 4 February 2018].

Detritus group in the different scenarios

For scenario 1, the detritus group included the total organic material in the sediment from phytoplankton and particulate organic matter from fish and mussel farms. The terrestrial input is assumed to be constant in all the scenarios.

Overnell and Young (1995) noted that the phytoplankton contribute to the total organic carbon in the sediment of upper Loch Linnhe at rates 0.082 g C/m^2/day, respectively. Using a conversion factor of 0.1 g C = 0.2 g dry weight = 1 g wet weight (Matthews and Heimdal 1980 cited in Mackinson and Daskalov 2007) the carbon input from phytoplankton in Loch Linnhe was estimated at 299.3 tonnes/km^2/year.

For scenario one, the detritus group contained a detritus of 300 tonnes/km^2/year from phytoplankton sources. Additionally, the group contained particulate waste (waste feed and faecal material) from fish farms and biodeposits from the mussel farm.

Fish farming contributes particulate waste towards the detritus group in the form of waste feed and uneaten faecal material. The total amount of feed for both years (2013 and 2014) and both farms (halibut and sea trout) was estimated at 359.5 tonnes/year. Assuming that 5% of the artificial feed is wasted (Gillibrand et al. 2002) the total amount wasted per year in the loch is estimated at 1.78 tonnes/km^2. Undigested feed from both fish farms was roughly estimated at 5.34 tonnes /km^2, assuming 15% of the feed is undigested (Gillibrand et al. 2002).

Mussels filter out food particles and small portion is used for physiological processes whereas a large portion of it is biodeposited as undigested deposits (pseudofaeces and faeces) (Wilding 2011; Pollet et al. 2015). Callier et al. (2009) reported that for 764 mussels/m^2 there is 16.8 g of biodeposits/m^2/d^2 and similar values were reported by Robert et al. (2013) where 200-400 mussels/m^2 biodeposited 4.4-8.8 g /m^2/day. For the model, I roughly estimated that if there are about 500 mussels/m^2 in the mussel farm area then the biodeposits would be 11 g /m^2/day. The estimated biodeposit distributed over the loch area was 7.95 tonnes/km^2 assuming approximately 10000000 mussels available over a 20000 m^2 area. The approximate number of mussels and the area were roughly estimated from the FARM model using a farm with measurments of 80 meters in width and 250 meters in length.

Total detritus for scenario 1 was estimated at a total of 315.1 tonnes/km^2. This includes detrital flow from phytoplankton (300 tonnes/km^2), fish farm waste (faeces and

undigested feed) of 7.12 tonnes/km^2 and biodeposits from the mussel farm of 7.95 tonnes/km^2.

For scenario 2, the total detritus was estimated at 307.1 tonnes/km^2 using 300 phytoplankton flows and 7.12 tonnes/km^2 for the fish farming waste (feed waste and faecal material).

For scenario 3, the total detritus was estimated at 308.0 tonnes/km^2 including phytoplankton flows and mussel farming biodeposits only (7.95 tonnes/km^2).

For scenario 4, the total detritus used was 300 tonnes/km^2 (phytoplankton sources only).

APPENDIX G

ADDITIONAL INFORMATION FOR ECOPATH MODEL SCENARIOS: CHAPTER 8.

This appendix provides supplementary information such as diet matrices and additional statistics output for scenarios 1-4 described in Chapter 8.

Table G.1 Diet composition matrix of the predator/prey (column/raw) in the model (presence of both aquaculture activities; scenario 1). The fraction of one compartment consumed by another is expressed as the fraction of the total diet. The sum of each column is equal to one.

	Prey\ predator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	Seabirds										
2	Mackerel	0.100									
3	Other fishes	0.480	0.620	0.050	0.050	0.031					
4	Juvenile whiting	0.120	0.010	0.010							
5	Crustaceans	0.100		0.150	0.150	0.079					
6	Echinoderms	0.080		0.150	0.150	0.150	0.060				
7	Zooplankton		0.220	0.440	0.150	0.108		0.042			
8	Polychaetes	0.100		0.150	0.150	0.150	0.190		0.036		
9	Farmed Fish	0.010									
10	Farmed Mussels	0.010				0.01	0.010				
11	Seaweed					0.150	0.150				
12	Phytoplankton					0.0425	0.250	0.825	0.570		0.900
13	Artificial Feed		0.150	0.025	0.300		0.010			0.800	
14	Detritus			0.025	0.05	0.280	0.340	0.133	0.394		0.100

Table G.2 Diet composition matrix of the predator/prey (column/raw) in the model scenario where only fish farming impacts on the Loch Melfort system considered (scenario 2). The fraction of one compartment consumed by another is expressed as the fraction of the total diet. The sum of each column is equal to one.

	Prey\ predator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Seabirds									
2	Mackerel	0.100								
3	Other fishes	0.490	0.620	0.050	0.050	0.031				
4	Juvenile whiting	0.120	0.010	0.01						
5	Crustaceans	0.100		0.150	0.150	0.078				
6	Echinoderms	0.080		0.150	0.150	0.155	0.060			
7	Zooplankton		0.220	0.440	0.150	0.118		0.042		
8	Polychaetes	0.100		0.150	0.150	0.155	0.190		0.036	
9	Farmed Fish	0.010								
10	Seaweed					0.150	0.150			
11	Phytoplankton					0.043	0.270	0.825	0.580	
12	Artificial Feed		0.150	0.025	0.300					0.800
13	Detritus			0.0025	0.050	0.270	0.330	0.133	0.384	

Table G.3 Diet composition matrix of the predator/prey (column/raw) in the model scenario where only mussel farming impacts on the Loch Melfort system were considered (scenario 3). The fraction of one compartment consumed by another is expressed as the fraction of the total diet. The sum of each column is equal to one.

	Prey\ predator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Seabirds									
2	Mackerel	0.100								
3	Other fishes	0.490	0.700	0.050	0.050	0.031				
4	Juvenile whiting	0.100	0.01	0.010						
5	Crustaceans	0.100		0.150	0.150	0.079				
6	Echinoderms	0.080		0.150	0.150	0.155	0.060			
7	Zooplankton		0.290	0.490	0.500	0.108		0.042		
8	Polychaetes	0.100		0.150	0.150	0.165	0.190		0.036	
9	Farmed Mussels	0.010				0.010	0.010			
10	Seaweed					0.150	0.150			
11	Phytoplankton					0.043	0.270	0.825	0.590	0.900
12	Detritus					0.260	0.320	0.133	0.374	0.100

Table G.4 Diet composition matrix of the predator/prey (column/raw) in the model scenario where no aquaculture impacts on the Loch Melfort system were considered (scenario 4). The fraction of one compartment consumed by another is expressed as the fraction of the total diet. The sum of each column is equal to one.

	Prey \ predator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	Seabirds								
2	Mackerel	0.100							
3	Other fishes	0.500	0.700	0.050	0.050	0.031			
4	Juvenile whiting	0.120	0.010	0.010					
5	Crustaceans	0.100		0.150	0.150	0.079			
6	Echinoderms	0.080		0.150	0.150	0.165	0.060		
7	Zooplankton		0.290	0.490	0.500	0.118		0.042	
8	Polychaetes	0.100		0.150	0.150	0.165	0.200		0.036
9	Seaweed					0.150	0.150		
10	Phytoplankton					0.043	0.280	0.825	0.664
11	Detritus					0.250	0.310	0.133	0.300

Table G.5 Comparison of different scenarios of the Loch Melfort ecosystem model and other ecosystems with fish and mussel farming.

	Model Scenarios				Other Ecosystems			
Parameters	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**	**Sardinia Island 1994 (before fish farming) (Díaz López et al. 2008)**	**Sardinia Island 2006 (after fish farming) (Díaz López et al. 2008)**	**Southeastern Spain (fish farming) (Bayle-Sempere et al. 2013)**	**Mont Saint Michel bay (mussel farming) (Leloup et al. 2008)**
Sum of all consumption (tonnes/km^2/year)	1167.645	1232.922	1083.713	1145.733	919.17	1912.91	31059.83	1090
Sum of all exports (tonnes/km^2/year)	116.068	96.316	97.604	78.224	110.35	267.29	23933.35	3700
Sum of all respiratory flows (tonnes/km^2/year)	724.254	758.842	691.581	724.555	294.40	677.47	13812.25	730
Sum of all flows into detritus (tonnes/km^2/year)	477.523	495.849	418.566	434.770	406.55	809.03	50795.5	3880
Total system throughput (TST) (tonnes/km^2/year)	2485.490	2583.929	2291.463	2383.281	1730	3667	119601	9400
Sum of all production (tonnes/km^2/year)	1008.123	1041.191	966.584	996.155	653	1232	12640	4570
Gross Efficiency (catch/net p.p.)	0.037	0.034	0.003	0.000		0.05	3.449	0.003
Calculated total net primary production (tonnes/km^2/year)	798.261	813.260	791.195	804.123	404.75	740.11	1604.96	4430
Total primary production/total respiration (TP/TR)	1.102	1.072	1.144	1.110	1.37	1.09	0.116	6.1
Net system production (tonnes/km^2/year)	74.007	54.418	99.614	79.568	110.34	62.63	-12207.29	3700

Total primary production/total biomass (TPP/TB)	6.797	6.482	8.487	7.992	7.79	4.61	0.204	24.6
Total biomass/total throughput (tonnes/km^2)	0.047	0.049	0.041	0.042	0.03	0.04	0.07	0.02
Total biomass (excluding detritus) (tonnes/km^2/year)	117.435	125.462	93.221	100.619	51.95	160.54	7864.55	180
Total catches	29.890	27.414	0.522	0.022		36.92	5535.78	15.9
Mean trophic level of the catch	2.001	2.001	2.015	2.715		2	2	2.11
Connectance index	0.357	0.380	0.364	0.390			0.19	0.17
System omnivory index	0.164	0.189	0.142	0.168	0.19	0.16	0.13	0.06
Finn's cycling index (FCI) (% of total throughput)	9.38	10.59	9.23	10.55	24.96	21.43		0.64
Finn's mean path length	2.680	3.021	2.903	2.968	4.27	3.88		2.10