The Influence of Sea Cage Aquaculture on Cage-associated Wild Fish

By © Luke McAllister

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Abstract

Sea cage aquaculture can alter the spatial distribution of wild fish populations; however, little is known about the dietary habits and subsequent effects on wild fish. In this thesis, I used stable isotopes $\delta^{13}C$ and $\delta^{15}N$ values and proportions of vegetable oil-based fatty acids (VOFAs) to investigate the dietary habits of wild cage-associated Atlantic cod *Gadus morhua* and Atlantic redfish *Sebastes fasciatus*. Furthermore, I compared the length, weight and condition of cage-associated *G. morhua* ages 2-4 years old to reference sites within the local division and outside divisions removed from aquaculture, and used VOFAs as biomarkers for waste feed consumption to identify any role(s) in explaining differences in length, weight and condition among age classes. Juvenile *G. morhua* captured around sea cages had depleted $\delta^{13}C$ and $\delta^{15}N$ values and elevated proportions of VOFAs consistent with waste feed consumption and sea cage residency, whereas differences in $\delta^{13}C$, $\delta^{15}N$, and VOFAs in cage-associated adult *G. morhua* and *S. fasciatus* were predominately absent. Interestingly, waste feed consumption by age 2 cage-associated *G. morhua* resulted in lower condition than age 2 *G. morhua* from the local reference division; however, age 4 cage-associated *G. morhua* were longer and heavier than the local reference division, despite no evidence to support direct or indirect waste-feed consumption. Overall, the results of this thesis suggest that $\delta^{13}C$, $\delta^{15}N$ and VOFAs are suitable biomarkers for tracing sea cage residency and dietary habits of cage-associated wild fish, but waste feed consumption patterns and subsequent effects on wild fish were paradoxical and dependent on species and life stage.

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I finally thank my mother for keeping me afloat through countless stressors, and of course my dogs, Dutch and his predecessor Molson for their unending love and emotional support which were invaluable during this process.

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List of Abbreviations and Symbols

ACP	Animal care protocol
ALA	α-linolenic acid (18:3ω3)
ARA	Arachidonic acid (20:4ω6)
ARC	Aquatic Research Cluster
BAME	Bacterial acid methyl ester
C : N ratio	Carbon-to-nitrogen ratio
^{43}Ca	Calcium-43
CCAC	Canadian Council on Animal Care
CREAIT	Core Research Equipment and Instrument Training
DFO	Fisheries and Oceans Canada
DHA	Docosahexaenoic acid (22:6ω3)
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (20:5ω3)
FA	Fatty acid
FAME	Fatty acid methyl ester
FCI	Fulton's condition index (*k*)
GC	Gas chromatography
GLM	General linear model
HIREC	Human induced rapid environmental change
IRMS	Isotope ratio mass spectrometer
LA	Linoleic acid (18:2ω6)
LA-ICP-MS	Laser ablation-inductively coupled plasma-mass spectrometry
LC-PUFA	Long-chain polyunsaturated fatty acid
MAF	Micro Analysis Facility
^{25}Mg	Magnesium-25
^{55}Mn	Mangenese-55
MUN	Memorial University of Newfoundland
NAFO	North Atlantic Fisheries Organization
NL	Newfoundland
OA	Oleic acid (18:1ω9)
OFI	Ocean Frontier Institute
PUFA	Polyunsaturated fatty acid
SIL	Stable Isotope Laboratory
^{88}Sr	Strontium-88
TDF	Trophic discrimination factor
TERRA	The Earth Resources Research and Analysis Facility
VOFA	Vegetable oil-based fatty acid
VPDB	Vienna PeeDee Belemnite
δ	Delta notation
Δ	Isotopic discrimination factor notation
$\delta^{13}C$	Delta carbon-thirteen
$\delta^{15}N$	Delta nitrogen-fifteen
ω3	Omega-3 fatty acid
ω6	Omega-6 fatty acid
ω6 : ω3	Omega-6 : omega-3 fatty acid ratio

Co-Authorship Statement

The research concept and framework presented within this thesis were developed by L.T. McAllister with assistance from my advisors, M.V. Abrahams and T.E. Van Leeuwen, and committee members C. Parrish and Y. Wiersma. Field and laboratory work were completed by L.T. McAllister with assistance from C. Conway and CREAIT and TERRA personnel including J. Potter, J. Wells and M. Wälle. Data analysis, thesis and manuscript(s) preparation were completed by L.T. McAllister with assistance from T.E. Van Leeuwen and M.V. Abrahams.

Chapter 1. General Introduction

1.1 Sea cage aquaculture and the association of wild fish

In Newfoundland (NL), marine finfish aquaculture production for Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* began in the mid-1980s at Bay d'Espoir and later expanded eastward into Fortune Bay (Pepper et al. 2003), with additional development in Placentia Bay (DFO 2022) and adjacent Connaigre Bay. Aquaculture production within NL produced less than a combined 20 t of *S. salar* and *O. mykiss* in 1986 (cited Rigby et al. 2017), however the industry peaked in 2016 and produced more than 25 000 t of predominately *S. salar* (Fisheries and Aquaculture 2021). Marine finfish aquaculture, for species including *S. salar* and *O. mykiss*, generally involves the hatching and development of juveniles in land-based facilities and then transport for rearing in open net sea cages, often along sheltered coastlines (Hvas et al. 2020). Marine aquaculture expansion has primarily occurred in coastal sea cages (Lin et al. 2019), which have been shown to influence local benthic communities (reviewed in Kalantzi & Karakassis 2006, Cullain et al. 2018), and alter the spatiotemporal distribution of wild fish populations across large scales (Giannoulaki et al. 2005, Goodbrand et al. 2013). Additionally, more than 160 species of wild fish have been shown to associate with sea cages (Sanchez-Jerez et al. 2011, Uglem et al. 2014), with wild fish aggregations observed in the vicinity of sea cages in Europe (Carss 1990, Dempster et al. 2002, 2010, Fernandez-Jover et al. 2011a,b, Gonzalez-Silvera et al. 2020), North America (Oakes & Pondella 2009), Australia (Dempster et al. 2004) and Asia (Sudirman et al. 2009). Dempster et al. (2009) estimated that wild fish aggregations were 1 to 3 orders of

magnitude higher around sea cages than control locations in Norway, and the majority of associated fish were located within 25 m of the cage structure (Dempster et al. 2010) for extended periods of time (Uglem et al. 2009). The phenomenon of wild fish association with cage structures appears similar to those observed for fish species with shipwrecks and offshore oil platforms (reviewed in Lee et al. 2018).

1.2 Mechanisms of wild fish attraction to sea cage aquaculture

Sea cages provide physical structure to wild fish and consequently a hypothesized meeting point to promote schooling behaviour (Dagorn & Fréon 1999, Soria et al. 2009) and increased shelter from predation (Beveridge 1984, Sanchez-Jerez et al. 2011). Additionally, 'spillover' of unconsumed aquafeed (i.e., waste feed), and potentially farm fish waste (reviewed in Uglem et al. 2014), can also serve as a direct food source and energetic subsidy for wild fish (Dempster et al. 2010, Sanchez-Jerez et al. 2011), or an indirect energetic subsidy through consumption of prey fish or benthic invertebrates that have been feeding on waste feed themselves (Dolenec et al. 2007, Bagdonas et al. 2012). Thus, according to optimal foraging theorems (see Stephens & Krebs 1987), waste feed should provide a predictable energetic subsidy that minimizes consumer foraging time and energetic expenditures (MacArthur & Pianka 1966, Scales et al. 2015), suggesting that even small quantities of waste feed should be highly profitable to cage-associated fish (Emlen 1966, Charnov 1976). Although, foragers must address additional physiological constraints, particularly balancing resource acquisition and risk of predation (Pulliam & Pyke 2007), with predation risk highly significant in forager behavioural decisions (Lima & Dill 1990). Furthermore, large concentrations of cage-associated prey

and juvenile fish (Serra-Llinares et al. 2013, Fernandez-Jover & Sanchez-Jerez 2015), closely associate with sea cages to reduce predation risk (Fernandez-Jover et al. 2009), inadvertently creating artificial hunting grounds fixed in space for predatory fish and marine mammals (Piroddi et al. 2011, Izquierdo-Gomez et al. 2014), easily discernable when compared to a featureless seascape (Cloern & Jassby 2008, 2010). Nevertheless, the production of aquafeed accounts for more than half of farm operation costs (reviewed in Emerenciano et al. 2013) and minimizing waste feed is a priority of the industry (Kolsäter 1995, Muñoz-Flores & Olivella-Nadal 2021). Technological monitoring and management (reviewed in Li et al. 2020), including subsurface camera monitoring systems (Parsonage & Petrell 2003), have allowed for more efficient and economical monitoring of aquafeed use; however, waste feed is still considered a significant attractant of wild fish to sea cages (Sanchez-Jerez et al. 2011). Regardless, even in the absence of waste feed, the mere structure availability of sea cages (i.e., cage, moorings) can alter spatial distribution patterns of wild fish (Goodbrand et al. 2013), and further supports a non-exclusive rationale(s) for cage association of wild fish aggregations (Sri & Kirubagaran 2015) that warrants further research (Callier et al. 2018).

1.3 Waste feed consumption and dietary habits of cage-associated wild fish

Despite the use of subsurface camera monitoring systems (Parsonage & Petrell 2003) to reduce aquafeed use, Uglem et al. (2014) estimated that thousands of tons of waste feed are accessible annually to wild fish in the vicinity of *S. salar* farms in Norway. However, the influence and consequences of waste feed consumption on cage-associated fish remains unclear. For example, waste feed consumption has been shown to increase the length, weight, and condition of wild cage-associated fish (Skog et al. 2003, Fernandez-Jover et al. 2007, Dempster et al. 2011), though decreased growth and size has also been observed for cage-associated juvenile fish species (Fernandez-Jover & Sanchez-Jerez 2015). Ontogeny-driven dietary habits are known to occur in cage-associated wild fish (Bagdonas et al. 2012), and particularly shifts from direct waste feed consumption by cage-associated juveniles (Sæther et al. 2012) to the consumption of farmed and associated fish aggregations (Sanchez-Jerez et al. 2008, Fernandez-Jover et al. 2009) by predatory and primarily adult wild fish aggregations (Dempster et al. 2002), suggesting the benefits of waste feed consumption and cage use could be dependent on species or life stage (Arechavala-Lopez et al. 2012). Nevertheless, the potential biomass increases associated with waste feed consumption (reviewed in Uglem et al. 2014) suggest that sea cages may act as a population source for cage-associated wild fish (Dempster et al. 2011), as observed in lake trout *Salvelinus namaycush* associating with freshwater aquaculture operations in Manitoba, Canada (Rennie et al. 2019).

1.4 Research relevance

The influence of sea cages on local wild fish remains poorly understood (Callier et al. 2018). Additionally, potential changes of local wild fish biomass and consequent fisheries availability (Machias et al. 2006, Uglem et al. 2014), are of particular interest in NL, given the large aggregations of wild fish that have been shown to occur in NL bays with sea cages (Goodbrand et al. 2013) and on-going stock recovery efforts for many commercial fish species (e.g., Atlantic cod *Gadus morhua*). Additionally, identifying the life history and biological traits in cage-associated wild fish, and eventually the underlying mechanisms that result in cage-association relative to species and life stage, is crucial to the successful management of cage-associated fish and the sustainability of the expanding aquaculture industry in NL.

1.5 Thesis outline

The research contained within this thesis investigated the dietary habits and biological effects of sea cage aquaculture on wild fish (i.e., length, weight, condition), to identify the underlying mechanisms that result in sea cage use and associated effects on wild fish, relative to species and life stage. In Chapter 2, I used wild juvenile Atlantic cod *Gadus morhua* reared in the laboratory, and fed either an aquafeed pellet or marine-based diet (squid), to determine the suitability of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ and vegetable oil-based fatty acids (VOFAs) to distinguish wild fish associated with sea cages, and determine waste feed consumption patterns of wild cage-associated *G. morhua* and Atlantic redfish *Sebastes fasciatus* and changes that occur with ontogeny. In Chapter 3, I used *G. morhua* collected in the direct vicinity of sea cages and reference divisions, either outside the vicinity of sea cages ('local division') or completely removed from aquaculture ('outside divisions'), to compare length, weight, and condition for cage-associated *G. morhua* ages 2-4 years old. Furthermore, I used proportions of VOFAs as biomarkers for waste feed consumption to determine its role in explaining differences in length, weight, and condition among cage-associated *G. morhua*. Finally, Chapter 4 provides a synopsis of all results and discusses their contribution to the understanding of sea cage use, dietary habits and traits (i.e., length, weight, condition) of cage-associated wild fish.

Chapter 2. Sea cage aquaculture may provide an energetic subsidy to wild juvenile Atlantic cod *Gadus morhua* in coastal bays of southern Newfoundland, Canada

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Abstract

Sea cage aquaculture can alter the spatial distribution of wild fish populations; however, little is known about the dietary habits of wild fish frequenting sea cages. I used wild juvenile Atlantic cod *Gadus morhua* reared in the laboratory and fed either an aquafeed pellet or marine-based diet to determine trophic discrimination factors (TDFs) of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in white muscle tissue and baseline liver vegetable oil-based fatty acid (VOFA) proportions. I then used $\delta^{13}C$ and $\delta^{15}N$ values and proportions of VOFAs to investigate the dietary habits of wild cage-associated juvenile and adult *G. morhua* and adult Atlantic redfish *Sebastes fasciatus*. *G. morhua* and *S. fasciatus* were collected in the immediate area of sea cages and reference areas of no aquaculture production. Juvenile *G. morhua* captured around sea cages had depleted $\delta^{13}C$ and $\delta^{15}N$ values, elevated proportions of VOFAs, and TDFs suggestive of sea cage use and similar aquafeed consumption to laboratory *G. morhua* fed an aquafeed diet. However, differences in $\delta^{13}C$ and $\delta^{15}N$ and proportions of VOFAs between cage-associated and reference site adult *G. morhua* and *S. fasciatus* were predominately absent. Results suggest that sea cages may provide an energetic subsidy to juvenile *G. morhua* but perhaps not at the level to sustain adult *G. morhua* or *S. fasciatus*. Therefore, the lack of differences suggests that both adult groups may be using sea cages opportunistically and only for short durations, as $\delta^{13}C$ and $\delta^{15}N$ values and proportions of VOFAs were not consistent with waste feed consumption, despite individuals being collected in close proximity to sea cages.

2.1 Introduction

Increasing demand for fish-derived animal protein has resulted in the rapid expansion of finfish aquaculture (Beveridge et al. 2013). Marine finfish aquaculture for species including Atlantic salmon *Salmo salar* generally involves the hatching and development of juveniles in land-based facilities and then transport to and rearing in open net sea cages, often in sheltered coastal bays (Hvas et al. 2020). Marine aquaculture expansion has primarily occurred in coastal sea cages (Lin et al. 2019) and has been shown to influence local biota abundances (reviewed in Cullain et al. 2018), similar to other artificial structures including shipwrecks and oil platforms (Lee et al. 2018, Bull & Love 2019). For example, more than 160 species of wild fish have been observed in the area of sea cages (Sanchez-Jerez et al. 2011, Uglem et al. 2014), and research suggests sea cages may alter the distribution of wild fish populations across large spatial extents (Giannoulaki et al. 2005, Dempster et al. 2009, Goodbrand et al. 2013).

Sea cages provide physical structure to wild fish and consequently a hypothesized meeting point to promote schooling behaviour (Dagorn & Freon 1999, Soria et al. 2009) and increased shelter from predation (Beveridge 1984, Sanchez-Jerez et al. 2011). 'Spillover' of unconsumed aquafeed, and potentially farm fish waste (reviewed in Uglem et al. 2014), also serves as a direct food source and energetic subsidy to wild fish (Dempster et al. 2010, Sanchez-Jerez et al. 2011) or an indirect energetic subsidy through consumption of prey fish or benthic invertebrates that have been feeding on excess aquafeed and farm fish waste themselves (Dolenec et al. 2007). Whatever the case, these hypotheses for wild fish association with sea cages are not exclusive (Sri & Kirubagaran

2015), and more research is necessary to determine how wild fish use sea cages (Callier et al. 2018) and if relationships change with life stage or species (Fernandez-Jover et al. 2009).

Many invertebrate and marine fish species possess complex life cycles that require the use of multiple habitats through ontogeny (Beck et al. 2001). For example, the use of inshore seagrass and submerged boulders as nursery habitats by juvenile fish (Gotceitas et al. 1997, Jackson et al. 2001, Auster et al. 2003, Lugendo 2007) and the use of deeper or offshore areas as adults (Cocheret de la Morinière et al. 2003) is well documented. Ontogenetic habitat shifts occur to limit overlap of resource use (Werner & Gilliam 1984) or as a response to ontogeny limiting the efficacy of their surroundings for protection from predation (Nagelkerken et al. 2000), and often to satisfy ontogenetic shifts in diet (Link & Garrison 2002) that can be detected using stable isotope signatures (Parker et al. 1989) deposited in fish muscle tissue (Pinnegar & Polunin 1999, MacNeil et al. 2005).

Consumer values of carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$) demonstrate consistent increases relative to the $\delta^{13}C$ values of their ambient environment (DeNiro & Epstein 1978, Sweeting et al. 2007b) and $\delta^{15}N$ values of their food sources (Minagawa & Wada 1984, Sweeting et al. 2007a), known as trophic discrimination factors (TDFs). Therefore, $\delta^{13}C$ and $\delta^{15}N$ can act as ecological tracers to reconstruct source carbon and thus determine habitat use (Nyunja et al. 2009, van Rijssel et al. 2016), as well as trophic structure (Post 2002, Choy et al. 2015) and dietary composition (MacNeil et al. 2005, Hussey et al. 2011). Sea cages produce unique $\delta^{13}C$ and $\delta^{15}N$ values in the surrounding water column (Xie et al. 2020), benthic communities (Karakassis et al. 2000, Aguado-

Gimenez & Ruiz-Fernandez 2012) and wild fish populations (Moreno Rojas et al. 2006, Jan et al. 2014), similar to unique $\delta^{13}C$ and $\delta^{15}N$ values used to distinguish biota in ecosystems surrounding urban pollution outflows (Hansson et. Al 1997, Mazumder et al. 2015).

Aquafeed fatty acid (FA) proportions may provide an additional ecological tracer (Dalsgaard et al. 2003), as aquafeed composition has higher proportions of vegetable oils (Turchini et al. 2010) that contain elevated proportions of carbon-18 (^{18}C) FAs including oleic acid (OA, 18:1ω9), linoleic acid (LA, 18:2ω6), and α-linolenic acid (ALA, 18:3ω3) and omega-6 (ω6) polyunsaturated FAs (PUFAs). Additionally, aquafeed is composed of lower proportions of omega-3 (ω3) PUFAs produced naturally by marine phytoplankton (Dalsgaard et al. 2003), and fish oil, which contains long-chain PUFAs (LC-PUFAs), such as docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3), that are necessary for optimal fish development (Sargent et al. 1999, Glencross 2009). Essential FAs (EFAs) cannot be synthesized *de novo* and must be acquired from dietary composition (Burr & Burr 1929, 1930) and includes LA (18:2ω6), ALA (18:3ω3) and DHA (22:6ω3) in many marine fish species (Holman 1986, Taşbozan & Gökçe 2017). Conditionally essential FAs are elongated carbon ω3 and ω6 LC-PUFAs (carbon length ≥ 20), including arachidonic acid (ARA, 20:4ω6) that can be synthesized in small quantities through necessary precursors but require additional dietary intake (Burke et al. 1999), and non-essential FAs such as OA (18:1ω9) that can be synthesized *de novo* (Caldari-Torres et al. 2016). Therefore, proportions of vegetable oil-based FAs (VOFAs) OA, LA and ALA and ω6 FAs in aquafeed should allow their use as biological tracers in FA profiles

of cage-associated fish (Skog et al. 2003, Fernandez-Jover et al. 2011a, Ramírez et al. 2013). The objectives of this study were to (1) determine the suitability of $\delta^{13}C$ and $\delta^{15}N$ values and proportions of VOFAs to distinguish wild fish associated with sea cages and (2) determine waste feed consumption of wild cage-associated *G. morhua* and *S. fasciatus* and changes that occur with ontogeny. I hypothesize that (1) laboratory feeding trials will allow for the development of TDFs and baseline FA proportions for the different diets, and (2) consumption of aquafeed in the laboratory and wild samples will result in unique $\delta^{13}C$ and $\delta^{15}N$ values and increased proportions of VOFAs including OA, LA and ALA, and ω6 FAs.

2.2 Methods

2.2.1 Laboratory experiment and rearing

Juvenile *G. morhua* were collected as part of a wider Fisheries and Oceans Canada (DFO) study by beach seine from Newman Sound and Placentia Bay (n = 36; Table 2.1, Fig. 2.1B) between October 17th-28th, 2019. Collected *G. morhua* were transported alive in coolers to the DFO wet laboratory in St. John's, Newfoundland (NL) per Canadian Council on Animal Care guidelines (CCAC 2010) and Memorial University of Newfoundland (MUN) Animal Care Protocol (ACP) no. 20200342, and distributed equally in 2 circular 1364 L tanks that were aerated and continuously supplied with flow-through seawater at ambient temperature and salinity from the nearby open coast. Tanks were cleaned daily and maintained on a simulated day : night cycle that mimicked ambient daylight for the duration of the treatments. All *G. morhua* were fed *ad libitum*

twice per week on 1 of 2 treatments: pieces of defrosted squid or seawater-moistened aquaculture pellets (Skretting), similar to those found falling through the cages at *S. salar* aquaculture sites. Samples of both squid and aquafeed were taken every other month, frozen and stored for comparisons between $\delta^{13}C$ and $\delta^{15}N$ values and VOFA proportions in the aquafeed pellet or squid and fish white muscle tissue. All laboratory *G. morhua* were fed either squid or herring *Clupea harengus* prior to diet treatments, depending on availability, and began treatments on January 10th, 2020 and were sacrificed on June 12th, 2020 for stable isotope and FA analysis after 155 d. Turnover of $\delta^{13}C$ and $\delta^{15}N$ in the muscle tissue of juvenile fish occurs rapidly, with half-life values of 14-25 days (Suzuki et al. 2005, Hamaoka et al. 2016), and liver FA proportions begin to reflect dietary alternative lipid sources in as little as 2 weeks (Skonberg et al. 1994), with a steady state reached in less than 4 months (Twibell et al. 2012). Therefore, the duration of treatments was considered sufficient for dietary stable isotope and FA assimilation.

2.2.2 Wild fish sample collection

Wild fish were separated into 'reference site' and 'cage-associated' groups based on collection location, with reference site *G. morhua* and *S. fasciatus* collected 50-530 km from sampled sea cages in Pools Cove, NL, and at a minimum of 10 km from any potential active or fallow sea cages. Reference samples were collected from further away than the distance known for sea cage particulates to be detected in $\delta^{13}C$ and $\delta^{15}N$ values (Sarà et al. 2004, Colombo et al. 2016) and VOFA proportions (White et al. 2017, Gonzalez-Silvera et al. 2020). Additionally, samples were collected at a distance further away than where a temporary association of wild fish with sea cages has been observed

(Uglem et al. 2009), and therefore were considered acceptable controls. Cage-associated *G. morhua* and *S. fasciatus* were collected in the immediate vicinity of sea cages (~ 10 m) to further afield at distances up to 300 m.

Reference site *G. morhua* were collected from Pouch Cove (n = 30, depth = 10-20 m; Table 2.1, Fig. 2.1B) by hook and line fishing between July 13th-27th, 2019, during the NL recreational groundfish season. Additional reference *G. morhua* and *S. fasciatus* samples were collected between October 31st-December 4th, 2018, and September 19th, 2019, during the annual DFO survey trawls from divisions 3Ps (n = 68, depth = 20-140 m; Table 2.1, Fig. 2.1B) and 3O (n = 48, depth = 192-618 m; Table 2.1, Fig. 2.1A). Furthermore, Robinson (2001) provided $\delta^{13}C$ and $\delta^{15}N$ values for juvenile *G. morhua* from Bonavista Bay (n = 19, depth = 10-25 m; Table 2.1, Fig. 2.1B) that were collected during their graduate studies. Cage-associated *G. morhua* (n = 76, depth = 15-100 m; Table 2.1, Fig. 2.1B) and *S. fasciatus* (n = 15, depth = 15-20 m; Table 2.1, Fig. 2.1B) were collected with permission from 5 locations in the immediate vicinity (~ 10 m) and further afield (≤ 300 m) of sea cages in Pools Cove (Table 2.1, Fig. 2.1B) by a combination of hook and line fishing and benthic longline between August 5th-8th, 2019. Once captured, all fish were euthanized by concussion (CCAC 2010, MUN ACP no. 20200342) and placed on ice or frozen until processing depending on the source of collection. Trawled fish were frozen and all other fish were placed on ice before sampling. In addition to whole fish collection, aquafeed samples (n = 3, ~ 10 g) were supplied directly from aquaculture companies operating near the sites of fish collection and frozen until analysis.

2.2.3 Preparation and analysis of stable isotope samples

White muscle tissue contains only small quantities of lipids and inorganic carbonates (Pinnegar & Polunin 1999), which reduces $\delta^{13}C$ and $\delta^{15}N$ variation observed in other tissues and yields the most predictable $\delta^{13}C$ and $\delta^{15}N$ values (Madigan et al. 2012). White muscle tissue was sampled (1.5 ± 0.5 g) from each fish at the anterior portion of the dorsum. Each muscle tissue sample was dried (VWR oven, 60ºC, 24-96 h) to remove water and reach a constant dried weight (Sherwood & Rose 2005). After being ground into a fine powder using a mortar and pestle cleaned with distilled water, 1.0 ± 0.1 mg of each dried sample powder was weighed (Mettler Toledo, AT21 Comparator) and transferred into 7 × 7 mm foil (Sn) capsules (Elemental Microanalysis, D5208), folded and stored for analysis.

For analysis, folded capsules were loaded into an elemental analyser (Carlos Erba Instruments, NA1500, N-Protein) attached to an isotope ratio mass spectrometer (IRMS; ThermoFinnigan, Delta V Plus) to determine $\delta^{13}C$ and $\delta^{15}N$ values in the Stable Isotope Laboratory (SIL) of the Core Research Equipment and Instrument Training (CREAIT) network at Memorial University of Newfoundland (MUN, St. John's). Isotope values are reported in the delta notation (δ), and Vienna PeeDee Belemnite (VPDB) is the internationally accepted reference standard for $\delta^{13}C$ measurements (Coplen 1995, Coplen et al. 2006) and air (0 ‰) or atmospheric nitrogen (N_2) is the internationally accepted reference standard for $\delta^{15}N$. Standards of USGS62 (caffeine) and B2155 (casein) were used to compare samples to accepted carbon and nitrogen isotope values and standard L-glutamic acid was used as a primer and for the elemental calibration of carbon and

nitrogen. Lipid correction of $\delta^{13}C$ was not necessary as the carbon-to-nitrogen (C : N) ratio of all samples was less than 3.5 and lipid content was considered negligible (Skinner et al. 2016). The experimental values of $\delta^{13}C$ and $\delta^{15}N$ for standards, USGS62, B2155 and L-glutamic acid were comparable to accepted values.

2.2.4 Lipid extraction

Liver samples (~ 250 mg) were removed from each individual fish and placed in pre-weighed 15 ml glass vials that were heated for 8 h at 450ºC. Liver tissue is considered more 'metabolically active' than white muscle tissue (Madigan et al. 2021) and has demonstrated more rapid isotopic and FA turnover than muscle tissue (MacNeil et al. 2005, 2006, Mohan et al. 2016). Samples were covered in 2 ml of chloroform ($CHCl_3$, ≥ 99.9%), and the tube headspace was filled with nitrogen (N_2, ≥ 99.9%), before being sealed with lipid-free caps (three times methanol [CH_3OH, ≥ 99.9%] and three times $CHCl_3$ washed). Samples were sealed with Teflon tape and stored at -20ºC until extraction. Lipid samples were extracted following Parrish (1999). Samples were homogenized in a 2 : 1 chilled $CHCl_3$: CH_3OH mixture with a Tissue Master 125 Homogenizer and a 7 mm Probe (Omni International). $CHCl_3$-extracted water was added to the chilled $CHCl_3$: CH_3OH solution to produce an 8 : 4 : 3 ratio mixture of $CHCl_3$: CH_3OH : water. Samples were sonicated in an ice bath for 4 min (Fisher Scientific, FS30H) and then centrifuged for 2 min at 1800 × *g* (Fisher Scientific, 74634H) causing the organic layer to separate from the aqueous layer. A double pipetting technique was used to remove the organic layer. A $CHCl_3$ rinse was then added to the extraction vial and the process repeated three times. The organic layer from each rinse was pooled in lipid-

cleaned vials for each sample (2 ml, 1.5 ml full). Nitrogen (N_2, ≥ 99.9%) was used to concentrate the samples and the vials capped and stored in a -20ºC freezer until gas chromatography (GC) analysis. Sample lipid extracts were transesterified for 1 h at 100ºC using sulfuric acid (H_2SO_4, ≥ 99.9%) and CH_3OH. The FA methyl esters (FAMEs) were analysed using a gas chromatograph system (Hewlett Packard, 6890, FID) with a 7683 autosampler in the Aquatic Research Cluster (ARC) at the Ocean Sciences Centre (OSC, St. John's). The gas chromatograph column (Phenomenex, ZB WAXplus) had a column length of 30 m and 0.32 mm internal diameter. The column was held at 65ºC for 30 s and then heated to 195ºC (40°C min^{-1}) and held for 15 min, then further heated to 220ºC (2ºC min^{-1}) and held for 45 s. Samples were injected using a hydrogen gas carrier (H_2) at a rate of 2 ml min^{-1}. The injector temperature of 150ºC was heated to 250ºC (120ºC min^{-1}) while the detector remained at 260ºC. Sample peaks were identified with retention times of Supelco 37 standards (Sigma Chemical): component FAME mix (47885-U), bacterial acid methyl ester mix (47080-U), PUFA 1 (47033) and PUFA 3 (47085-U). A quantitative standard (Nu-Chek prep, GLC490) was used after around 300 samples to test chromatograph FID accuracy. Chromatographs of fatty acid profiles were then processed (Agilent OpenLAB Data Analysis – Build 2.203.0.573).

2.2.5 Calculations and statistical analyses

2.2.5a TDF calculation

Discrimination of $\delta^{13}C$ and $\delta^{15}N$ in diet source to sample muscle tissue was used to determine dietary habits of fish captured in the vicinity of cages and to compare laboratory TDFs to established values (DeNiro & Epstein 1978, Minagawa & Wada 1984):

$$TDF = I_{muscle} - I_{diet}$$

where TDF is the isotopic discrimination from diet source to sample muscle tissue provided in Δ notation, I_{muscle} is the baseline isotopic value of muscle tissue and I_{diet} is the baseline isotopic value of diet source both provided in δ notation.

2.2.5b Laboratory experiment

I tested for the effects of group (laboratory *G. morhua* fed an exclusively aquafeed diet and laboratory *G. morhua* fed an exclusively squid diet), fork length and the interaction between group and fork length on sample values of $\delta^{13}C$ and $\delta^{15}N$ using general linear models (GLMs). Proportions of VOFAs (OA, LA and ALA) and the ω6 : ω3 ratio were also compared using GLMs with the same factors described above for the $\delta^{13}C$ and $\delta^{15}N$ analysis. Additionally, group proportions of ω6 and ω3 FAs, ARA and DHA were compared using GLMs and are provided in the appendices (Table 2.1S, Fig. 2.1S).

2.2.5c Wild fish caught in the area of sea cage sites and reference sites

I tested for the effects of group (cage-associated fish and reference site fish), species (*G. morhua* and *S. fasciatus*), length and the interaction effects of group × species, group × length, species × length and the three-way interaction effect of group × species × length on $\delta^{13}C$ and $\delta^{15}N$ values using GLMs. Further, I compared proportions of VOFAs (i.e., OA, LA, ALA) and the ω6 : ω3 ratio using GLMs with the same factors described above for the $\delta^{13}C$ and $\delta^{15}N$ analysis, and group proportions of ω6 and ω3 FAs, ARA and DHA using GLMs that are provided (Table 2.1S, Fig. 2.1S). An effect of capture location was tested on reference group values of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ and proportions of VOFAs, and the ω6 : ω3 ratio to account for natural variations in stable isotope and FA biomarkers. Fork length was used as a covariate in the stable isotope models to control for known length effects on diet for *G. morhua* (Link & Garrison 2002, Hanson 2011, van Leeuwen et al. 2013) and the genus *Sebastes* (Lambert 1960, González et al. 2000) and compounded $\delta^{13}C$ and $\delta^{15}N$ values caused by trophic transfer from larger fish feeding at higher trophic levels (Hobson & Welch 1992, Vizzini et al. 2002). Because of a significant interaction between species and group, I split and modelled the isotope and FA values for *G. morhua* and *S. fasciatus* separately. These GLMs contained group, length and a group × length interaction. Further, because of a significant interaction between group and length in these models, the use of both juveniles and adults in our *G. morhua* data set and clear patterns in ontogenetic changes in diet type of *G. morhua* between juveniles and adults, the *G. morhua* data was further split into juveniles (length ≤ 45 cm) and adults (length ≥ 46 cm) to ensure isotopic and FA differences were not simply a result

of a known ontogenetic switch at this length between invertebrate feeding as juveniles and piscivorous feeding as adults (Hanson 2011). The resulting stable isotope models contained group and length as fixed effects whereas the FA models only contained group as a fixed effect. If a significant factor was found a Tukey's post hoc test using the package emmeans (Lenth 2020, v. 1.5.1) in R was used when necessary to compare levels within a factor. Assumptions of homogeneity of variances and normality were checked using a Bartlett's test and a Shapiro-Wilks test, respectively. Statistical analyses were completed using R statistical software (R Core Team 2019, v. 3.6.0).

2.3 Results

2.3.1 Laboratory experiment

2.3.1a Stable isotopes and TDFs

Laboratory-reared *G. morhua* fed an exclusively aquafeed diet had $\delta^{13}C$ and $\delta^{15}N$ TDFs of 2.13 ± 0.25 and 6.57 ± 0.21‰ (mean ± SE; Fig. 2.2) and laboratory-reared *G. morhua* fed an exclusively squid diet had $\delta^{13}C$ and $\delta^{15}N$ TDFs of 2.61 ± 0.09 and 1.70 ± 0.19‰, respectively (Fig. 2.2). Aquafeed pellets used in the laboratory experiment had a significantly higher $\delta^{13}C$ ($F_{1,4} = 37.65$, $p < 0.01$; Table 2.2) and C : N ratio ($F_{1,4} = 17.02$, $p = 0.01$; Table 2.2) but significantly lower $\delta^{15}N$ ($F_{1,4} = 968.51$, $p < 0.001$; Table 2.2) than the squid diet. Therefore, as expected, laboratory-reared *G. morhua* fed an exclusively aquafeed diet had significantly higher $\delta^{13}C$ (Table 2.3, Fig. 2.3A) but significantly lower $\delta^{15}N$ (Table 2.3, Fig. 2.3A) than laboratory-reared *G. morhua* fed an exclusively squid diet.

2.3.1b FA proportions

Aquafeed pellets contained 1200% higher proportions of OA ($F_{1,2} = 6592.3$, $p < 0.001$; Table 2.4, Fig. 2.4A), 2600% higher proportions of LA ($F_{1,2} = 7437.1$, $p < 0.001$; Table 2.4, Fig. 2.4B), 1900% higher proportions of ALA ($F_{1,2} = 1920.8$, $p < 0.001$; Table 2.4, Fig. 2.4C) and a 3200% higher ω6 : ω3 ratio ($F_{1,2} = 1459.2$, $p < 0.001$; Table 2.4, Fig. 2.4D) than the squid diet. This resulted in laboratory-reared *G. morhua* fed an exclusively aquafeed diet having 240% higher proportions of OA (Table 2.5, Fig. 2.4A), 1000% higher proportions of LA (Table 2.5, Fig. 2.4B), 400% higher proportions of ALA (Table 2.5, Fig. 2.4C) and an 1100% higher ω6 : ω3 ratio (Table 2.5, Fig. 2.4D) than laboratory-reared *G. morhua* fed an exclusively squid diet.

2.3.2 Cage-associated and reference site wild fish: effects of species and ontogeny

2.3.2a Stable isotopes and TDFs

Cage-associated juvenile *G. morhua* had $\delta^{13}C$ and $\delta^{15}N$ TDFs of 3.19 ± 0.09 and 9.46 ± 0.20‰ (Fig. 2.2) and *S. fasciatus* collected from sea cage sites had $\delta^{13}C$ and $\delta^{15}N$ TDFs of 3.09 ± 0.05 and 9.84 ± 0.07‰ (Fig. 2.2), whereas adult *G. morhua* collected from sea cage sites had $\delta^{13}C$ and $\delta^{15}N$ TDFs of 3.85 ± 0.24 and 11.43 ± 0.30‰ (Fig. 2.2). Aquafeed pellets supplied by aquaculture companies at the site of fish collection had significantly lower $\delta^{13}C$ ($F_{1,4} = 161.81$, $p < 0.001$; Table 2.2) and significantly lower $\delta^{15}N$ ($F_{1,4} = 4851$, $p < 0.001$; Table 2.2) than squid samples used in the laboratory study, and a lower $\delta^{13}C$ and $\delta^{15}N$ than known *G. morhua* and *S. fasciatus* prey items including capelin (*Mallotus villosus*), northern prawn (*Pandalus borealis*) and Arctic eualid (*Eualus*

fabricii) from NL (Sherwood & Rose 2005; Table 2.2). Cage-associated juvenile *G. morhua* had significantly lower $\delta^{13}C$ and $\delta^{15}N$ than reference site juvenile *G. morhua* (Table 2.3, Fig. 2.3B) but there was no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between adult *G. morhua* collected from sea cage sites and reference sites (Table 2.3, Fig. 2.3C). No significant differences between *S. fasciatus* groups were found in $\delta^{13}C$ (Table 2.3, Fig. 2.3D) but cage-associated *S. fasciatus* had significantly higher $\delta^{15}N$ than reference site *S. fasciatus* (Table 2.3, Fig. 2.3D).

2.3.2b FA proportions

Aquafeed pellets, supplied by aquaculture companies at the site of fish collection, had 1600% higher proportions of OA ($F_{1,3} = 10449$, $p < 0.001$; Table 2.4, Fig. 2.4A), 3200% higher proportions of LA ($F_{1,3} = 9923.7$, $p < 0.001$; Table 2.4, Fig. 2.4B), 3000% higher proportions of ALA ($F_{1,3} = 5392.2$, $p < 0.001$; Table 2.4, Fig. 2.4C) and a 4700% higher ω6 : ω3 ratio ($F_{1,3} = 3398$, $p < 0.001$; Table 2.4, Fig. 2.4D) than the squid diet used in the laboratory feeding experiment, and much higher than known prey items in the wild (Kirsch et al. 1998, Copeman & Parrish 2004, Parrish et al. 2012, Olsen et al. 2015; see Table 2.4). Juvenile *G. morhua* collected from sea cages had significantly higher proportions of OA (Table 2.5, Fig. 2.4A), LA (Table 2.5, Fig. 2.4B) and ALA (Table 2.5, Fig. 2.4C) and a higher ω6 : ω3 ratio (Table 2.5, Fig. 2.4D) than juvenile *G. morhua* collected from reference sites. Cage-associated adult *G. morhua* had significantly higher OA proportions than adult *G. morhua* collected from reference sites (Table 2.5, Fig. 2.4A) but no significant differences were found between groups for proportions of LA (Table 2.5, Fig. 2.4B), ALA (Table 2.5, Fig. 2.4C) or the ω6 : ω3 ratio (Table 2.5, Fig.

2.4D). There were no significant differences between *S. fasciatus* collected from sea cage sites and reference sites for proportions of OA (Table 2.5, Fig. 2.4A), LA (Table 2.5, Fig. 2.4B) or ALA (Table 2.5, Fig. 2.4C), or the ω6 : ω3 ratio (Table 2.5, Fig. 2.4D).

2.4 Discussion

The depleted $\delta^{13}C$ and $\delta^{15}N$ values and enriched proportions of VOFAs in cage-associated juvenile *G. morhua* allowed for the separation of cage-associated and reference site fish by stable isotope and FA analysis. Proportions of essential, conditionally essential and nonessential FAs, and $\delta^{13}C$ and $\delta^{15}N$ TDFs in cage-associated juvenile *G. morhua* indicate direct and indirect feeding on sea cage site waste feed, as determined based on values obtained in my laboratory feeding experiment, and suggest that cage-associated juvenile *G. morhua* may be receiving an energetic subsidy from sea cage aquaculture sites. However, the limited differences (i.e., adult *G. morhua* OA proportions, *S. fasciatus* $\delta^{15}N$ values) between adult *G. morhua* and *S. fasciatus* collected from sea cage sites and references sites requires interpretation, as adults of both species were caught in close proximity to sea cage sites during the study. One interpretation could be the waste feed subsidy provided to smaller juvenile *G. morhua* may not be sufficient to sustain larger adult *G. morhua* or *S. fasciatus*, and thus a size threshold exists whereby larger individuals increase consumption of prey at higher trophic levels. This explanation is plausible as isotopic and stomach content research on NL *G. morhua* and *Sebastes* spp. have shown ontogeny-driven diet shifts resulting in consumption of prey at higher trophic levels (González et al. 2000, Sherwood et al. 2007) and fewer instances of waste feed observed in stomach samples of cage-associated adult fish compared to juvenile fish

(Sæther et al. 2012). In addition, the increased mobility and foraging range of adult *G. morhua* and *S. fasciatus* (Petitgas et al. 2013, Sullivan et al. 2017) may reduce periods of cage-association to timeframes that are insufficient to assimilate stable isotope values and FA proportions of a sea cage environment; however, *G. morhua* and *S. fasciatus* affinity to certain habitats, often including physical structure (Auster et al. 2003, Staveley et al. 2019), may result in sporadic or short-term use of cages as observed in Norway (Uglem et al. 2008).

Stable isotope values and FA proportions of cage-associated juvenile *G. morhua* observed in my study are consistent with differences in $\delta^{13}C$, $\delta^{15}N$, OA, LA, ALA and the ω6 : ω3 ratio found in previous studies investigating consumption of waste feed in wild fish species (Skog et al. 2003, Moreno Rojas et al. 2006, Fernandez-Jover et al. 2007, Jan et al. 2014, Gonzalez-Silvera et al. 2016). Calculated $\delta^{13}C$ and $\delta^{15}N$ TDFs for cage-associated juvenile *G. morhua* and cage-associated *S. fasciatus* were greater than a single trophic discrimination (DeNiro & Epstein 1978, Minagawa & Wada 1984) but lower than cage-associated adult *G. morhua*, indicating that cage-associated juvenile *G. morhua* and *S. fasciatus* fed at a similar trophic level, and may partially consume waste feed indirectly at a trophic level that is lower than that of cage-associated adult *G. morhua*. $\delta^{13}C$ and $\delta^{15}N$ TDFs for laboratory *G. morhua* were comparable to those found in previous research (Sweeting et al. 2007a,b) as well as for aquaculture *S. salar* in NL (Dempson & Power 2004). However, C : N ratios and consequently lipid content of squid and lab aquafeed samples differed significantly from cage aquafeed, and TDFs could not be compared between diets. FA proportions can identify dietary and habitat use relationships

that are not reflected in $\delta^{13}C$ and $\delta^{15}N$ values (Olsen et al. 2015, Sardenne et al. 2020) and stable isotope and FA analysis are considered complementary ecological indicators (Woodcock et al. 2018). Cage-associated juvenile *G. morhua* had lower proportions of OA, LA, ALA and a lower ω6 : ω3 ratio compared to laboratory *G. morhua* fed an aquafeed diet, and when combined with stomach contents, may indicate a mixed diet of aquafeed pellet and primary consumers, including cage-associated invertebrates or zooplankton (Fernandez-Jover et al. 2016).

Animals select habitat to maximize their net rate of energy intake and minimize their mortality rate (Gilliam & Fraser 1987), and habitat is most often selected by juvenile fish to satisfy requirements of shelter, food and protection from predation (Heck et al. 2003). Sea cages provide a fixed structure and consistent feed subsidy to cage-associated fish, thereby reducing consumer search time and energy output, while providing an energetic subsidy to cage-associated fish, that in turn, may be linked to increased body condition and energy reserves (Fernandez-Jover et al. 2007, Dempster et al. 2011). For example, Rennie et al. (2019) found that increased energy reserves resulted in earlier sexual maturity, larger size at maturity and higher growth rates for cage-associated lake trout *Salvelinus namaycush*. This research would suggest cage-associated juvenile *G. morhua* in my study are likely using sea cage habitat to take advantage of the physiological benefits of reduced prey search time and capture time associated with waste feed in sea cage environments.

The results of previous studies investigating waste feed sedimentation on surrounding ecosystems have been mixed and observations range from reduced benthic diversity (Brown et al. 1987, Karakassis et al. 2000) to no discernible effect (Tanner & Williams 2015), or increased benthic diversity (reviewed in Kalantzi & Karakassis 2006), but nevertheless, direct waste consumption by cage-associated juvenile fish, as demonstrated in my study, could reduce the environmental impact of waste feed benthic sedimentation (Kutti et al. 2007). Juvenile *G. morhua* are generalist feeders, and site-enriched invertebrates may provide an additional trophic resource to cage-associated juvenile fish (Dolenec et al. 2007). Sea cages also provide shelter from predation to small fish (Beveridge 1984) and samples that were collected in the immediate vicinity of sea cages (~ 10 m) were exclusively juvenile (≤ 45 cm), similar to the findings of Fernandez-Jover et al. (2009), who suggested that juvenile fish may remain in close proximity to the cage structure to reduce predation risk, as observed in natural nursery habitats including sea grass and cobblestone (Gotceitas & Brown 1993, Fraser et al. 1996, Lilley & Unsworth 2014). However, predation risk was not quantified in my study and remains to be tested.

Habitat requirements change with development (Gunter 1967), and marine fish often occupy different juvenile and adult habitats (reviewed in Beck et al. 2001). Similarly, my results suggest that wild fish sea cage use is dependent on species and life stage. Cage-associated adult *G. morhua* $\delta^{13}C$ and $\delta^{15}N$ values and FA proportions (excluding OA) and *S. fasciatus* $\delta^{13}C$ values and FA proportions were comparable to reference site $\delta^{13}C$ and $\delta^{15}N$ values and FA proportions. Furthermore, $\delta^{13}C$ and $\delta^{15}N$

values of adult *G. morhua* and *S. fasciatus* were comparable to $\delta^{13}C$ and $\delta^{15}N$ values of NL fish reported by Sherwood & Rose (2005), suggesting that these individuals were consuming a more 'natural diet' consisting of increased proportions of ω3 FAs, DHA and ARA biosynthesized by marine plants, algae and phytoplankton (Dalsgaard et al. 2003), and only trace proportions of VOFAs. Therefore, the results of this study suggest that sea cage use by adult *G. morhua* and *S. fasciatus* is influenced at least partially by reasons besides direct waste feed consumption, or perhaps that site use is relatively short and waste feed consumption is limited.

Stable isotope values and FA proportions were consistent with a few exceptions, namely cage-associated *S. fasciatus* $\delta^{15}N$ values and elevated OA proportions in cage-associated adult *G. morhua*. Sea cage effluent has been demonstrated in previous studies to enrich entire coastal ecosystem $\delta^{15}N$ food webs (reviewed in Dolenec et al. 2007), and despite *S. fasciatus* being collected varying distances from sea cages, altered $\delta^{13}C$ and $\delta^{15}N$ values and VOFA proportions have been detected in crabs and brittle stars up to 1 km from cages (Woodcock et al. 2018) and up to 2.5 km from sea cages in other cage-associated invertebrates (Colombo et al. 2016). Therefore, sea cages may enrich invertebrate or sessile prey, as observed elsewhere (Redmond et al. 2010, Colombo et al. 2016), which are then consumed by cage-associated *S. fasciatus*. In addition, natural fluxes in environmental $\delta^{15}N$ values (Robinson 2001, Pereira et al. 2007) may have contributed to elevated *S. fasciatus* $\delta^{15}N$ values. Essential and conditionally essential FAs must be acquired through the diet and large proportions of LA, ALA and ω6 FAs in aquafeed, but trace proportions in marine environments allows their use as biological

tracers for primary consumption, though multiple dietary and FA sources can lower their applicability in tracing secondary and tertiary consumption (Auel et al. 2002, Dalsgaard et al. 2003). However, elevated proportions of OA are common in marine consumers (Falk-Petersen et al. 1990), and elevated OA proportions in cage-associated adult *G. morhua* may be attributed to increased prey aggregations in the area of sea cages (Serra-Llinares et al. 2013), including juvenile *G. morhua* that may have been feeding on waste feed themselves. In addition, OA composed the largest FA proportion of sea cage aquafeed samples and assimilates quickly into fish muscle (Skonberg et al. 1994) with an extended 'washout' period (Regost 2001, Robin et al. 2003). Adult *G. morhua* from the local reference division had elevated OA proportions, relative to the additional control groups, which could suggest an effect of location and may explain the elevated OA proportions in cage-associated adult *G. morhua*. Nevertheless, stable isotopes $\delta^{13}C$ and $\delta^{15}N$ values, VOFA proportions (OA, LA, ALA) and the ω6 : ω3 FA ratio provide insight into habitat use and dietary habits of cage-associated fish, but sea cage use of wild fish may vary seasonally (Dempster et al. 2002) and further study on a larger temporal scale is needed.

Future research areas should focus on individual biological changes, community changes and patterns of wild fish association with sea cages. Aquafeed-derived energetic subsidies have been linked to increases in condition and growth of laboratory *G. morhua* (Olsen et al. 2015), as well as improved survival, faster maturation and reproduction in cage-associated fish (Rennie et al. 2019), and altered growth and weight observed in sessile cage-associated organisms (Handå et al. 2012, White et al. 2018). Composition and abundances of surrounding benthic communities are also significantly impacted by sea cage aquaculture (Kalantzi & Karakassis 2006, Terlizzi et al. 2010, Cullain et al. 2018), as well as pelagic communities (Papastamatiou et al. 2010, Arechavala-Lopez et al. 2013).

2.5 Conclusion

Although more than 160 species of wild fish have been observed in the vicinity of sea cages (Sanchez-Jerez et al. 2011), the understanding of wild fish association with sea cages has been primarily hypotheses-driven (Sri & Kirubagaran 2015), and additional studies are necessary (Callier et al. 2018). In conclusion, I suggest that habitat use of sea cages is non-exclusive, and sea cage dietary patterns of wild fish are dependent on species and life stage.

2.6 Tables

Table 2.1 Northwest Atlantic Fisheries Organization (NAFO) divisions of wild fish collection, species collected (Atlantic cod *Gadus morhua* and Atlantic redfish *Sebastes fasciatus*), sample size (n) and fork length (mean ± SE).

Collection sites	Species	n	Length
Study site (cage-associated)			
Pools Cove	*G. morhua*	76	32.0 ± 1.2
	S. fasciatus	15	27.5 ± 0.5
Reference sites			
Pouch Cove	*G. morhua*	30	54.6 ± 2.0
NAFO 3Ps	*G. morhua*	68	50.3 ± 1.9
NAFO 3O	*G. morhua*	8	38.3 ± 1.8
	S. fasciatus	40	26.4 ± 0.6
Bonavista Bay	*G. morhua*	19	31.9 ± 1.3
Laboratory collection			
Newman Sound-Placentia Bay	*G. morhua*	36	19.3 ± 0.4

Table 2.2 Sample groups and muscle tissue isotope values (mean ± SE) of carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$) of laboratory-reared Atlantic cod *Gadus morhua* fed an aquafeed (pellet fed) or squid diet (squid fed) and the aquafeed (lab pellet) and squid fed in the lab (squid), and cage-associated (study site) *G. morhua* and Atlantic redfish *Sebastes fasciatus* and cage aquafeed (cage pellet). Literature $\delta^{13}C$ and $\delta^{15}N$ values of common *G. morhua* and *S. fasciatus* prey (Sherwood & Rose 2005) are provided and the carbon-to-nitrogen (C : N) ratio when applicable (lab pellet, squid, cage pellet).

Groups	$\delta^{13}C_{tissue}$	$\delta^{15}N_{tissue}$	Feed C : N
Laboratory *G. morhua*			
Pellet fed	-18.27 ± 0.25	13.18 ± 0.21	-
Squid fed	-19.16 ± 0.09	14.75 ± 0.19	-
Lab pellet	-20.40 ± 0.22	6.61 ± 0.18	5.78 ± 0.23
Squid	-21.77 ± 0.00	13.05 ± 0.10	4.85 ± 0.02
Study site			
Juvenile *G. morhua*	-19.47 ± 0.10	13.27 ± 0.20	-
Adult *G. morhua*	-19.10 ± 0.17	15.33 ± 0.41	-
S. fasciatus	-19.61 ± 0.05	13.62 ± 0.08	-
Cage pellet	-22.70 ± 0.07	3.78 ± 0.09	7.77 ± 0.14
Reference sites			
Juvenile *G. morhua*	-19.06 ± 0.07	14.94 ± 0.16	-
Adult *G. morhua*	-19.28 ± 0.09	15.30 ± 0.15	-
S. fasciatus	-19.62 ± 0.10	13.04 ± 0.14	-
Literature prey			
Capelin *Mallotus villosus*	-21.0 ± 0.10	12.2 ± 0.09	-
Northern prawn *Pandalus borealis*	-18.2 ± 0.12	11.4 ± 0.30	-
Arctic eulid *Eualus fabricii*	-18.9 ± 0.02	14.3 ± 0.43	-

Table 2.3 General linear modelling (GLM) results for isotope values (‰) of carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$) of Atlantic cod *Gadus morhua* and Atlantic redfish *Sebastes fasciatus* collected from sea cage sites and reference sites, and laboratory-reared juvenile *G. morhua* fed an aquafeed or squid diet. Initial models contained group (G), length (L), species (S) and their interactions (G × L, G × S, S × L and G × L × S).

Models	Fixed Effects	df	n	F	p
Laboratory cod $\delta^{13}C$		2, 13	16	3.48	< 0.05
	Group	1		4.81	< 0.05
	Length	1		0.13	0.73
Laboratory cod $\delta^{15}N$		2, 13	16	27.53	< 0.001
	Group	1		19.76	< 0.001
	Length	1		11.51	< 0.01
All samples $\delta^{13}C$		3, 129	133	15.41	< 0.001
	Group	2		17.42	< 0.001
	Length	1		18.07	< 0.001
All samples $\delta^{15}N$		7, 125	133	24.41	< 0.001
	Group	2		12.04	< 0.001
	Length	1		22.79	< 0.001
	Species	1		4.22	0.04
	G × S	1		33.74	< 0.001
	G × L	2		12.54	< 0.001
Atlantic cod $\delta^{13}C$		3, 100	104	6.10	< 0.001
	Group	1		5.51	0.02
	Length	1		0.32	0.57
	G × L	1		10.01	< 0.01
Atlantic cod $\delta^{15}N$		3, 100	104	35.46	< 0.001
	Group	1		30.21	< 0.001
	Length	1		23.30	< 0.001
	G × L	1		16.27	< 0.001
Juvenile cod $\delta^{13}C$		3, 74	78	6.55	< 0.001
	Group	1		11.43	< 0.01
	Length	1		0.11	0.74
	G × L	1		5.53	0.02
Juvenile cod $\delta^{15}N$		2, 75	78	28.48	< 0.001
	Group	1		26.60	< 0.001
	Length	1		11.03	< 0.01
Adult cod $\delta^{13}C$		3, 22	26	3.80	0.12
	Group	1		4.30	< 0.05
	Length	1		0.14	0.71
	G × L	1		6.72	0.02
Adult cod $\delta^{15}N$		2, 23	26	0.14	0.76
	Group	1		0.10	0.76
	Length	1		0.22	0.64
Redfish δ^{13}		2, 26	29	8.74	0.72
	Group	1		0.13	0.72
	Length	1		17.47	< 0.001
Redfish $\delta^{15}N$		2, 26	29	6.64	0.01
	Group	1		7.50	0.01
	Length	1		4.45	0.04

Table 2.4 Sample groups and liver fatty acid (FA) proportions (mean ± SE) of oleic acid (OA, 18:1ω9), linoleic acid (LA, 18:2ω6), α-linolenic acid (ALA, 18:3ω3) and the omega-6 : omega-3 FA (ω6 : ω3) ratio for laboratory-reared Atlantic cod *Gadus morhua*, lab pellet and squid, cage-associated (study site) *G. morhua* and Atlantic redfish *Sebastes fasciatus*, cage pellet, and literature FA proportions of common *G. morhua* and *S. fasciatus* prey (Kirsch et al. 1998, Copeman & Parrish 2004, Parrish et al. 2012).

Groups	OA	LA	ALA	ω6 : ω3
Laboratory *G. morhua*				
Pellet fed	30.70 ± 3.13	10.37 ± 1.49	2.20 ± 0.29	0.69 ± 0.11
Squid fed	12.78 ± 0.45	0.98 ± 0.11	0.54 ± 0.08	0.06 ± 0.00
Lab pellet	32.15 ± 0.05	12.35 ± 0.10	3.10 ± 0.03	0.98 ± 0.02
Squid	2.64 ± 0.36	0.46 ± 0.10	0.16 ± 0.06	0.03 ± 0.00
Study site				
Juvenile *G. morhua*	23.15 ± 1.48	5.44 ± 0.69	1.53 ± 0.17	0.47 ± 0.06
Adult *G. morhua*	23.08 ± 1.01	2.62 ± 0.75	0.66 ± 0.02	0.22 ± 0.04
S. fasciatus	22.79 ± 3.44	1.13 ± 0.16	0.41 ± 0.07	0.69 ± 0.11
Cage pellet	42.99 ± 0.22	14.80 ± 0.10	4.90 ± 0.04	1.42 ± 0.02
Reference sites				
Juvenile *G. morhua*	12.90 ± 0.96	0.98 ± 0.03	0.49 ± 0.14	0.12 ± 0.02
Adult *G. morhua*	12.87 ± 0.73	1.12 ± 0.04	0.77 ± 0.18	0.10 ± 0.00
S. fasciatus	25.09 ± 1.74	1.28 ± 0.20	0.39 ± 0.05	0.06 ± 0.00
Literature prey				
N. shortfin squid *Illex Illecebrosus*	3.4 ± 0.08	0.40 ± 0.01	0.30 ± 0.01	0.05 ± 0.00
Blue mussel *Mytilus edulis*	1.1 ± 0.06	1.40 ± 0.06	0.90 ± 0.06	0.10 ± 0.05
Copepod *Calanus finmarchicus*	2.2 ± 0.12	0.60 ± 0.12	0.14 ± 0.12	0.03 ± 0.00

Table 2.5 General linear modelling (GLM) results for fatty acid (FA) proportions (percentage of FA profile) of oleic acid (OA, 18:1ω9), linoleic acid (LA, 18:2ω6), α-linolenic acid (ALA, 18:2ω6) and the omega-6 : omega-3 FA (ω6 : ω3) ratio of Atlantic cod *Gadus morhua* and Atlantic redfish *Sebastes fasciatus* collected from sea cage sites and reference sites, and laboratory-reared juvenile *G. morhua* fed an aquafeed diet or squid diet. Initial models contained group (G), length (L), species (S) and their interactions (G × L, G × S, S × L and G × L × S).

Models	Fixed Effects	Df	n	F	p
OA – 18:1ω9					
Laboratory *G. morhua*	Group	1, 12	14	25.39	< 0.001
Juvenile *G. morhua*	Group	1, 57	59	9.25	< 0.01
Adult *G. morhua*	Group	1, 21	23	6.86	0.02
S. fasciatus	Group	2, 24	27	0.83	0.37
	Length			6.12	0.02
LA – 18:2ω6					
Laboratory *G. morhua*	Group	1, 12	14	28.80	< 0.001
Juvenile *G. morhua*	Group	1, 57	59	8.20	< 0.01
Adult *G. morhua*	Group	1, 21	23	0.22	0.64
S. fasciatus	Group	1, 25	27	0.22	0.65
ALA – 18:3ω3					
Laboratory *G. morhua*	Group	1, 12	14	23.21	< 0.001
Juvenile *G. morhua*	Group	1, 57	59	7.68	< 0.01
Adult *G. morhua*	Group	1, 21	23	0.06	0.81
S. fasciatus	Group	1, 25	27	0.06	0.82
ω6 : ω3 ratio					
Laboratory *G. morhua*	Group	1, 12	14	25.07	< 0.001
Juvenile *G. morhua*	Group	1, 57	59	7.23	< 0.01
Adult *G. morhua*	Group	1, 21	23	0.64	0.43
S. fasciatus	Group	1, 25	27	0.56	0.46

2.7 Figures

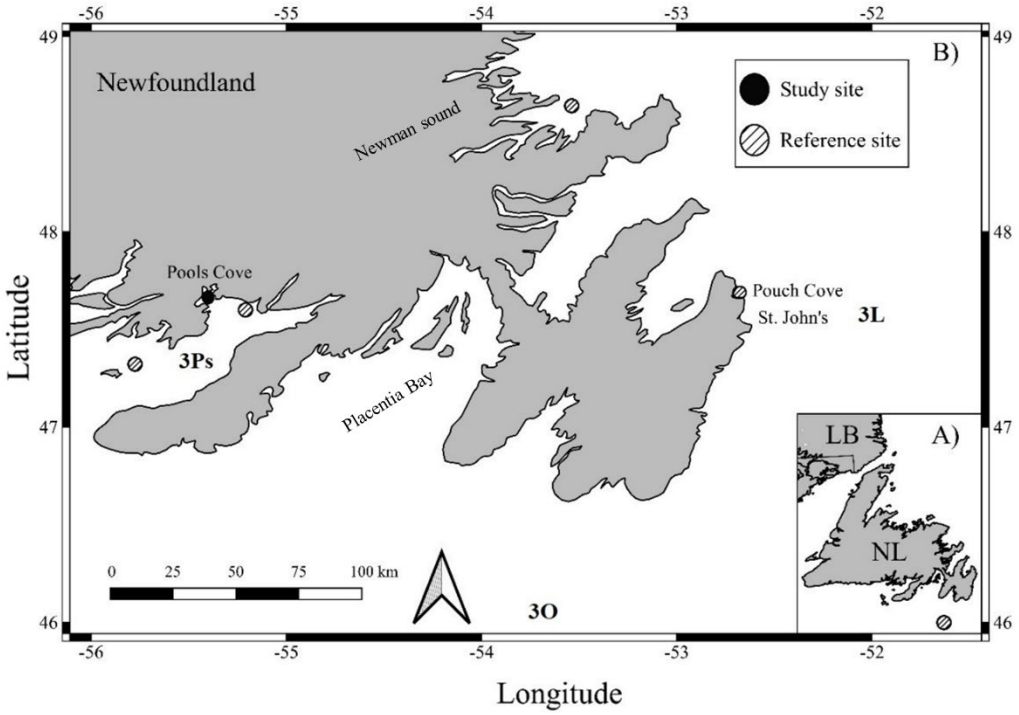

Figure 2.1 (A) Overview of Newfoundland, Canada and Northwest Atlantic Fisheries Organization (NAFO) 3O sampling location and (B) locations of additional wild Atlantic cod *Gadus morhua* and Atlantic Redfish *Sebastes fasciatus* sample collection sites. Aquaculture cage-associated *G. morhua* and *S. fasciatus* were collected with permission in the direct vicinity of aquaculture sea cages in Pools Cove (study site). Sites of collections with no immediate (< 10 km) aquaculture production (reference sites) included Pouch Cove, Bonavista Bay and NAFO division 3O and 3Ps. Laboratory-reared *G. morhua* were collected in Newman Sound and Placentia Bay, Newfoundland, Canada.

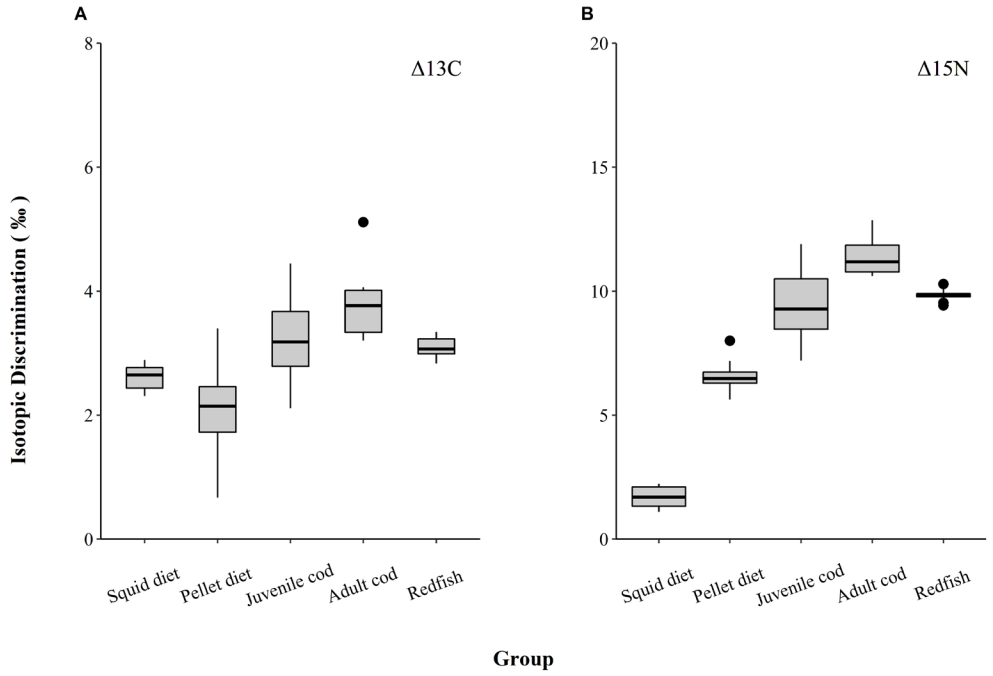

Figure 2.2 (A) Muscle tissue carbon-13 (δ^{13}C) trophic isotope discrimination (‰) of laboratory-reared juvenile Atlantic cod *Gadus morhua* compared to an aquafeed pellet (pellet diet) and squid (squid diet) source δ^{13}C and study site (cage-associated) juvenile *G. morhua* (cod), adult *G. morhua* (cod) and Atlantic redfish *Sebastes fasciatus* (redfish) compared to study site cage pellet source δ^{13}C and (B) nitrogen-15 (δ^{15}N) trophic isotope discrimination values (‰) of laboratory-reared juvenile *G. morhua* compared to an aquafeed pellet (pellet diet) and squid (squid diet) source δ^{15}N, and study site (cage-associated) juvenile *G. morhua* (cod), adult *G. morhua* (cod) and *S. fasciatus* (redfish) compared to study site cage pellet source δ^{15}N. Boxplots represent group median (horizontal line), 75th percentile (top of grey box) and 25th percentile (bottom of grey box), minimum value (lower grey 'whisker'), maximum value (upper grey 'whisker') and individual outliers (black circle).

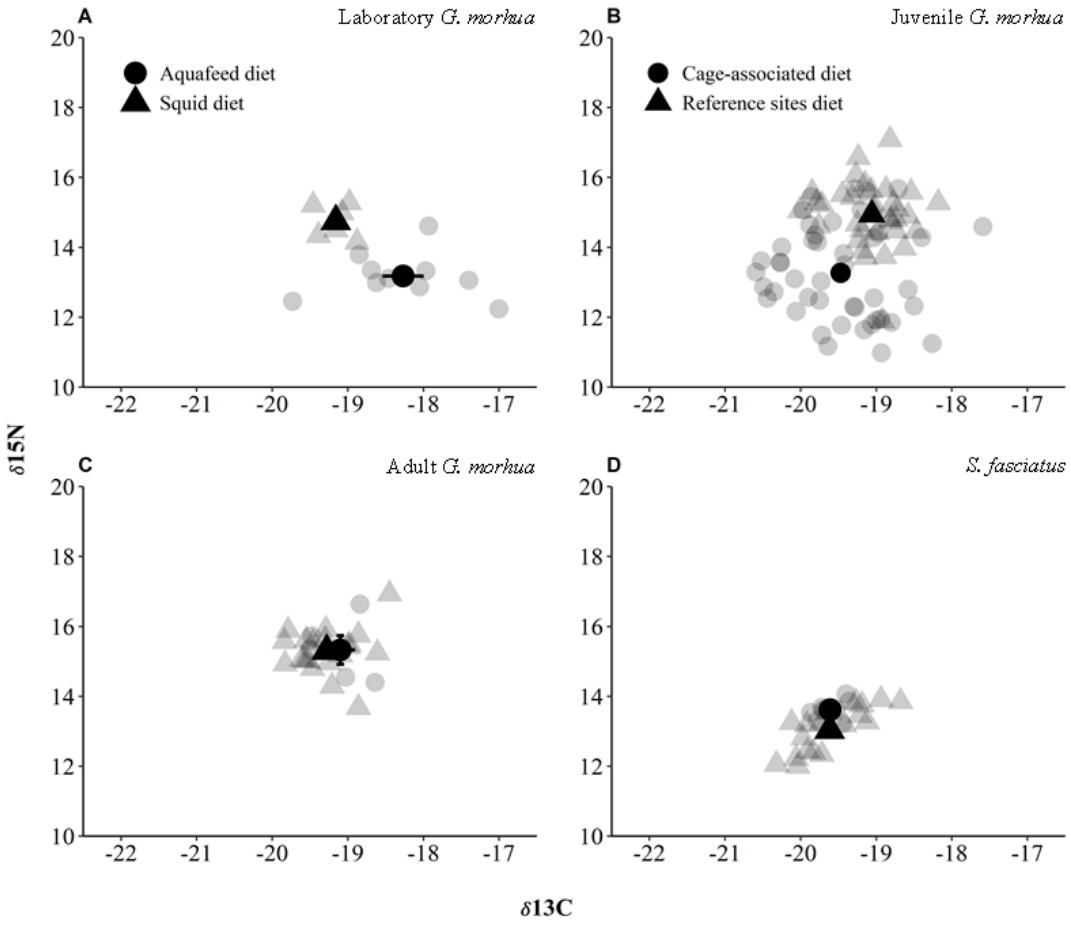

Figure 2.3 Mean (± SE) muscle tissue $\delta^{13}C$ and $\delta^{15}N$ values (black circle, triangle respectively) and individual $\delta^{13}C$ and $\delta^{15}N$ values (grey circle, triangle respectively) for (A) juvenile laboratory-reared Atlantic cod *Gadus morhua* fed either an aquafeed diet or squid diet and (B) juvenile *G. morhua*, (C) adult *G. morhua* and (D) Atlantic redfish *Sebastes fasciatus* (*S. fasciatus*) collected from sea cage sites with a cage-associated diet or reference sites with a reference site ('natural') diet.

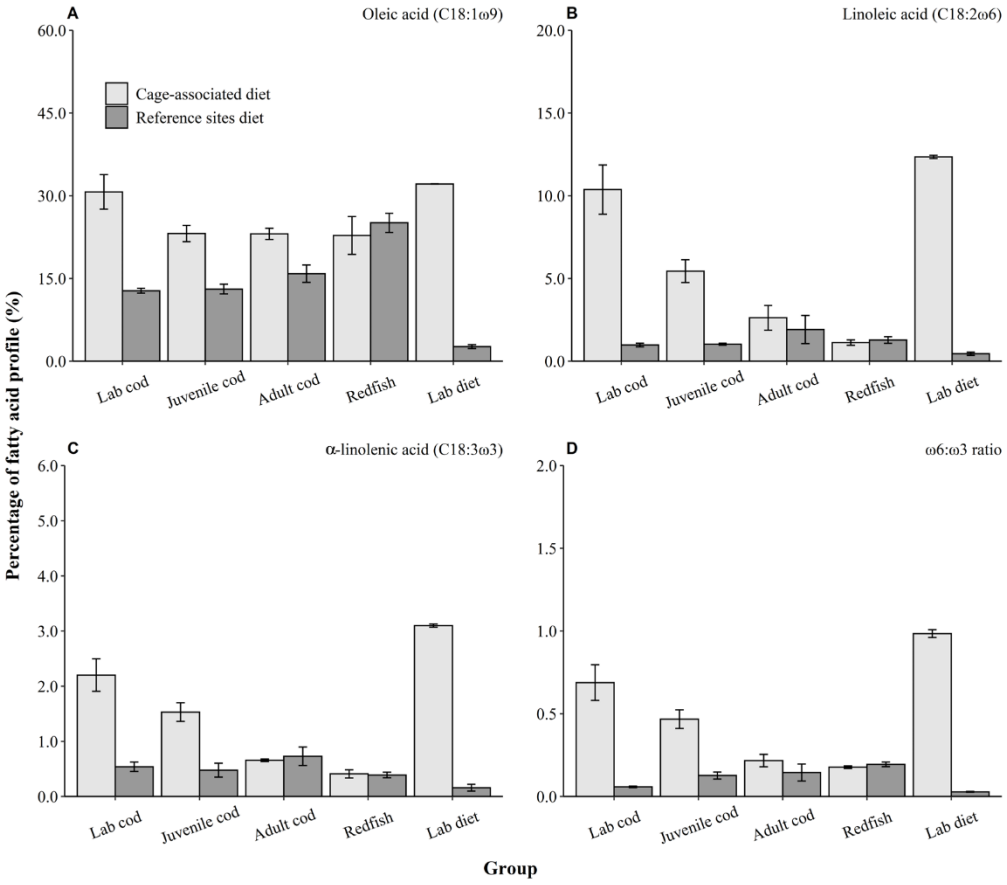

Figure 2.4 Mean (± SE) liver proportions of (A) oleic acid (OA 18:1ω9), (B) linoleic acid (LA, 18:2ω6), (C) α-linolenic acid (ALA 18:3ω3) and (D) the omega-6 : omega-3 FA (ω6 : ω3) ratio expressed as a percentage of fatty acid profiles for laboratory *G. morhua* fed either an aquafeed diet or squid diet and juvenile *G. morhua*, adult *G. morhua* and *S. fasciatus* collected from sea cage sites and reference sites. Reference sites included Pouch Cove and NAFO divisions 3O and 3Ps, Newfoundland, Canada.

Chapter 3. The effect of sea cage aquaculture on the length, weight, and condition of wild Atlantic cod *Gadus morhua*

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Abstract

Wild fish have been shown to associate with sea cage aquaculture sites and consume waste feed; however, little is known about the effects of waste feed consumption on wild fish. I used Atlantic cod *Gadus morhua* collected in the direct vicinity of sea cages and reference divisions, either outside the direct vicinity ('local division') or completely removed from aquaculture ('outside divisions'), to compare length, weight, and condition for *G. morhua* ages 2-4 years old. Concentrations of vegetable oil-based fatty acids (VOFAs) were then used as biomarkers for waste feed consumption to determine their role in explaining differences in length, weight, and condition among cage-associated *G. morhua*. Age 2 cage-associated *G. morhua* were in lower condition than age 2 *G. morhua* from the local division and lighter than age 2 *G. morhua* from all outside divisions. Age 3 cage-associated *G. morhua* were comparable to age 3 *G. morhua* from the local division, but in lower condition than age 3 *G. morhua* from all outside divisions. However, age 4 cage-associated *G. morhua* were longer and heavier than age 4 *G. morhua* from the local division. Additionally, there were positive relationships between fatty acid concentrations and length and weight for age 2 cage-associated *G. morhua*, but no such relationships for age 3 or 4 *G. morhua*. Results suggest the effects of waste feed consumption may be paradoxical but likely not large enough to disrupt growth and condition related patterns among surrounding populations.

3.1 Introduction

Open net sea cages, such as those used to farm Atlantic salmon *Salmo salar* along sheltered coastlines (Hvas et al. 2020), can attract and aggregate wild fish species across large spatial scales (Dempster et al. 2004, Giannoulaki et al. 2005, Goodbrand et al. 2013), and increase abundances of local wild fish (Machias et al. 2004, 2005). The physical structure of the cage and moorings may provide a common 'meeting point' for wild fish (Dagorn & Fréon 1999, Fréon & Dagorn 2000) and facilitate schooling, hunting, and shelter-seeking behaviours (Beveridge 1984, Soria et al. 2009, Izquierdo-Gomez et al. 2014). Additionally, unconsumed waste aquafeed, and potentially farm fish feces (reviewed in Uglem et al. 2014), can be consumed directly and indirectly, through consumption of cage-associated prey fish, benthic invertebrates, and zooplankton (Sanchez-Jerez et al. 2008, Fernandez-Jover et al. 2009) that routinely consume waste feed themselves (Sæther et al. 2012, Callier et al. 2013, Fernandez-Jover et al. 2016). Therefore, regardless of consumption route, waste feed should provide a predictable energetic subsidy that minimizes consumer foraging time and energetic expenditures (Scales et al. 2015), thus according to optimal foraging theorems (see Stephens & Krebs 1987) even small quantities of waste feed should be highly profitable to cage-associated fish and result in increased growth and condition (Skog et al. 2003, Fernandez-Jover et al. 2007). However, additional factors such as life stage, competition, and predation rates also require consideration (Fretwell & Lucas 1969, Dahlgren & Eggleston 2000, Craig & Crowder 2002, Bartolino et al. 2011), and could affect sea cage habitat quality and associated benefits to wild fish.

Increased proportions of vegetable oil-based fatty acids (VOFAs) associated with waste feed composition, particularly linoleic acid (LA, 18:2ω6) and α-linolenic acid (ALA, 18:3ω3), are essential fatty acids (EFAs) that assimilate into cage-associated pelagic and benthic communities (Fernandez-Jover et al. 2009, Woodcock et al. 2018, 2019), but are unable to be synthesized *de novo* in marine fish species (Tocher 2003, Wu & Chen 2012). Therefore, given their rapid assimilation and elongated retention times (Torstensen et al. 2004, Olsen et al. 2015), and natural rarity in marine ecosystems (Dalsgaard et al. 2003, White et al. 2019), LA and ALA have been shown to be suitable biomarkers for tracing waste feed consumption in cage-associated wild fish (Arechavala-Lopez et al. 2011, Abaad et al. 2016, White et al. 2019, McAllister et al. 2021).

I used wild Atlantic cod *Gadus morhua* collected in the direct vicinity of sea cages and reference divisions, either outside the direct vicinity of sea cages or completely removed from aquaculture to (1) compare length, weight, and condition (Fulton's index, *k*) for *G. morhua* ages 2-4 years old and (2) identify the role, if any, of waste feed consumption in explaining differences in length, weight, and condition among cage-associated *G. morhua*. Assuming that waste feed represents a significant energetic subsidy not available to non cage-associated *G. morhua* populations (Dempster et al. 2011), I hypothesize that (1) *G. morhua* collected from sea cages will be longer, heavier and in better condition than *G. morhua* collected from reference sites and (2) increased LA and ALA concentrations (i.e., biomarkers of waste feed consumption) will result in positive correlations with length, weight, and condition in younger cage-associated *G. morhua* (2-3 years old), but correlations will be reduced or non-existent in older *G.*

morhua (4 years old) due to an ontogeny-linked reduction in waste feed consumption and dependency (McAllister et al. 2021).

3.2 Methods

3.2.1 Wild fish sample collection

Wild *G. morhua* were separated into 'cage-associated' and 'reference' groups based on collection location. Cage-associated *G. morhua* were collected between August 5th-8th 2019 from the immediate vicinity (10-300 m) of sea cages in Pools Cove, Newfoundland (NL), Canada (n = 72; Table 3.1, Fig. 3.1) with permission, using a combination of hook and line fishing with a rod and benthic longline per Canadian Council on Animal Care guidelines (CCAC 2010) and Memorial University of Newfoundland (MUN) Animal Care Protocol (ACP) no. 20200342. Reference *G. morhua* were collected from multiple sampling trips that were completed between March 31st-December 18th 2019, from sites either outside the immediate vicinity of sea cages (> 10 km), hereafter referred to as 'local division' (3Ps: n = 329, March 31st-December 18th; Table 3.1, Fig. 3.1) or completely removed from *S. salar* aquaculture, hereafter referred to as 'outside divisions' (3L: n = 546, May 28th-November 26th; 3N: n = 245, May 16th-October 15th; 3O: n = 162, May 4th-September 27th; Table 3.1, Fig. 3.1). Sampling trips included Fisheries and Oceans Canada (DFO) survey trawls and beach seines. Once captured, *G. morhua* were euthanized by concussion (CCAC 2010, MUN ACP no. 20200342) and either placed on ice or frozen prior to measurements of fork length and

round weight, and the removal of liver tissue (≥ 250 mg), gonads, and sagittal otoliths for fatty acid analysis, sexing, and aging, respectively.

3.2.2 Correction for a common sampling date

Given the extensive spatial coverage of sampling it was impossible to collect all reference *G. morhua* on the same date as the cage-associated *G. morhua*. Therefore, length, weight, and condition indices for reference *G. morhua*, from both the local division and outside divisions, were adjusted to the sampling month in which the cage-associated *G. morhua* were collected (August), to ensure that any effects were not simply due to sampling period (Mello & Rose 2005). This was done for length and weight, using a linear regression between measurements that were collected from the spring and summer and at least one additional month during either the fall and winter seasons (Lambert & Dutil 1997), for both the local division and outside divisions and for each age class (ages 2-4 years old; see Table 3.2S for regression slope, intercept and R-squared values). The percent change in monthly averages were then applied to each sample from the local division and outside divisions (Parrish & Mallicoate 1995). Corrected length and weight values were used to calculate Fulton's condition index (FCI), as a general measurement of fish condition (reviewed in Nash et al. 2006), where W = seasonally corrected fish weight (g), and L = seasonally corrected fish length $(cm)^3$. FCI values > 1 indicate above average condition, whereas FCI values < 1 indicate below average condition.

3.2.3 Thin section preparation and sample aging

3.2.3a Thin section preparation

Sagittal otoliths provide accurate age measurements for teleost fish (reviewed in Campana 2001) and are most commonly used for microstructure analysis (Campana & Neilson 1985). Sample otoliths were removed from *G. morhua* brain cavities and rinsed, and the right otolith from each fish were embedded (Stuers, 25: 3 epoxy : hardener), cured overnight and sectioned (Buehler, Isomet low-speed saw) to identify the core. Four blades (0.5 mm), separated by spacers (0.65 mm), were used to obtain the otolith core (S. Campana, personal correspondence). Thin sections were then polished in 10 s intervals (Gator, 800 grit sanding cloth) to improve microstructure and growth ring clarity (DFO 2019) and photographed (Nikon, SMZ1500).

3.2.3b Aging (visual and laser ablation inductively coupled plasma mass spectrometry)

Aging was conducted using two methods, visually and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). For visual aging, sectioned annuli rings (i.e., thin sections) were identified under a microscope, using offsetting summer (opaque) and winter (hyaline) zones and a standard northern hemisphere birth date of January 1st. A double-blind review was then completed with a trained secondary reader. LA-ICP-MS was conducted using the ablation process and equipment detailed in D'Avignon & Rose (2013). Briefly, *G. Morhua* thin sections were fixed horizontally and pre-ablated (10 Hz repetition rate). An ultraviolet light excimer laser ablation system (Coherent, GeoLasHD) with a wavelength of 193 nm was then used to ablate transects

(core-edge), using an energy density of 4 J / cm^2, a scanning speed of 20 µm s^{-1} and a spot size of 50 µm. The laser ablation system was coupled to an inductively coupled plasma mass spectrometer (Thermo Fisher, Element XR), which measured elemental concentrations (ppm mg/kg^{-1}), of magnesium (^{25}Mg), manganese (^{55}Mn) and strontium (^{88}Sr), using ^{43}Ca as an internal standard (40%) and National Institute of Standards and Technology 610 as an external standard and United States Geological Society MACS-1 for quality control. Seasonal changes in abiotic conditions (i.e., salinity, temperature, oxygen availability) and metabolic activity result in consistent shifts in otolith magnesium, manganese, and strontium elemental concentration ratios (^{88}Sr : ^{25}Mg, ^{25}Mg : ^{43}Ca and ^{55}Mn : ^{43}Ca) and are considered applicable for aging (reviewed in Heimbrand et al. 2020). Therefore, seasonal variation pattern peaks in ^{88}Sr : ^{25}Mg, ^{25}Mg : ^{43}Ca and ^{55}Mn : ^{43}Ca, were identified, counted and used to determine *G. morhua* age (Hüssy et al. 2016).

All *G. morhua* collected during the DFO survey trawls and beach seines were aged visually under a microscope (n = 1282), whereas all cage-associated *G. morhua* were aged using a combination of microscopy (n = 72) and LA-ICP-MS (n = 30). Of the 30 thin sections aged with both techniques, no discrepancy in aging was found. Elemental concentrations were determined in the Micro Analysis Facility of the Core Research Equipment and Instrument Training network at Memorial University of Newfoundland.

3.2.4 Lipid biomarker extraction

Waste feed consumption can be detected through lipid-based LA (18:2ω6) and ALA (18:3ω3) concentrations, which were extracted following Parrish (1999) and detailed in Chapter 2 (2.2.4 Lipid extraction). Briefly, liver samples (~ 250 mg) were removed from each fish and placed in 15 ml glass vials that were pre-weighed and heated to degrade any contaminants (muffle furnace, 8 hrs, 450ºC). Samples were covered in 2 ml of chloroform ($CHCl_3$, ≥ 99.9%) and the tube headspace was filled with nitrogen (N_2, ≥ 99.9%) before being sealed with lipid-cleaned caps (methanol: [CH_3OH, ≥ 99.9%] and $CHCl_3$ wash repeated three times) and stored at -20ºC until lipid extraction. For extraction, samples were homogenized (Omni International Inc., Tissue Master 125) using a 7 mm probe in a chilled $CHCl_3$: CH_3OH (2 : 1) mixture. $CHCl_3$-extracted water was then added to produce a $CHCl_3$: CH_3OH : water (H_2O) (8 : 4 : 3) mixture. Samples were sonicated for 4 min (Fisher Scientific, FS30H) using an ice bath, centrifuged for 2 min at 1800 x *g* (Fisher Scientific, 74634H), and then the organic layer was removed by a double-pipetting technique. A chloroform rinse was completed using an extraction vial with each organic layer pooled in lipid-cleaned vials (2 ml, 1.5 ml full) and repeated three times. Organic layers were then concentrated with nitrogen, capped and sealed, and stored at -20ºC until gas chromatography (GC) analysis.

For GC analysis, samples were transesterified for 1 h at 100ºC using sulfuric acid (H_2SO_4, ≥ 99.9%) and CH_3OH to produce fatty acid methyl esters (FAMEs), that were then analyzed using a gas chromatograph (HP, 6890) with a 7683 autosampler. The gas chromatograph column (Phenomenex USA, ZB WAXplus) with a 30 m length and 0.32

mm internal diameter was heated to 65ºC for 30 s and 195ºC (40ºC min^{-1}) for 15 minutes and 220ºC (2ºC min^{-1}) for 45 s. Samples were injected (~ 1.5 ml by volume) with hydrogen (H_2) as the carrier gas at 2 ml min^{-1} using an injector temperature of 150ºC that was heated to 250ºC (120ºC min^{-1}) and a detector temperature of 260ºC. Sample peaks were identified using retention times for standards (Sigma Chemical, Supelco 37): component FAME mix (47885-U), BAME mix (47080-U), PUFA 1 (47033) and PUFA 3 (47085-U) and chromatograph FID accuracy was tested with a quantitative standard (Nu-Chek prep, GLC490) after approximately 300 samples. Chromatographs of lipid profiles were then developed (Agilent OpenLAB Data Analysis, Build 2.203.0.573).

3.2.5 Calculations and statistical analyses

I tested for the effects of group (cage-associated, local division, outside divisions), age (2, 3, 4 years), sex, and the interactions between group × age, group × sex, and the three-way interaction of group × age × sex on collection month-adjusted sample values for length, weight, and condition using general linear models (GLMs). Given a significant interaction between group and age, separate GLMs were developed for length, weight, and condition for each age class with group and sex as fixed effects. Further, I used separate GLMs for each age class to test for the effect of LA and ALA concentrations on length, weight, and condition values of cage-associated *G. morhua*. For all models, assumptions of homogeneity of variances and normality were checked prior to analyses using a Bartlett's test and a Shapiro-Wilks test, respectively. Statistical analyses were completed using R statistical software (R Core Team 2021, v. 4.1.1).

3.3 Results

3.3.1 Length, weight, and condition comparisons

There was no significant difference ($p \geq 0.05$) in length and weight between the cage-associated *G. morhua* and the local division for age 2 and 3 *G. morhua* (Table 3.2, Fig. 3.2A,B), though age 2 cage-associated *G. morhua* were approximately 27 g lighter on average than those from the local reference division ($p = 0.07$; Table 3.1). However, cage-associated age 2 *G. morhua* had a significantly lower condition than age 2 *G. morhua* from the local division, whereas age 3 cage-associated *G. morhua* were of similar condition to age 3 *G. morhua* from the local division (Table 3.2, Fig. 3.2C). Lastly, age 4 cage-associated *G. morhua* were more than 3 cm longer and nearly 200 g heavier on average than *G. morhua* from the local division (Table 3.2, Fig. 3.2A,B) but of similar condition (Table 3.2, Fig. 3.2C).

To determine whether length, weight, and condition patterns were large enough to transcend those of surrounding populations, cage-associated *G. morhua* were also compared to *G. morhua* from three outside divisions. Results of this analysis were highly variable among traits and age classes, with only age 2 cage-associated *G. morhua* being significantly lighter (~ 20 g on average) than age 2 *G. morhua* from all outside divisions (Table 3.2, Fig. 3.2B) and age 3 cage-associated *G. morhua* having a significantly lower condition than age 3 *G. morhua* from all outside divisions (Table 3.2, Fig. 3.2C).

3.3.2 LA and ALA concentrations vs. length, weight, and condition relationships

There was a significant positive relationship between LA / ALA and length and weight for age 2 cage-associated *G. morhua* (Table 3.3, Fig. 3.3A,B), but no significant relationships between LA / ALA and length and weight for age 3 and 4 *G. morhua* (Table 3.3, Fig. 3.3A,B). Similarly, there was no significant relationship between LA / ALA and condition across all ages of cage-associated *G. morhua* (Table 3.3, Fig. 3.3C).

3.4 Discussion

When the length, weight, and condition of cage-associated *G. morhua* were compared to those from the outside divisions, the weight of age 2 cage-associated *G. morhua* and condition of age 3 cage-associated *G. morhua* were the only traits significantly different from all outside divisions. Thus, one may conclude that the effects of *S. salar* aquaculture on wild *G. morhua* were large enough to suppress weight and condition. However, the lower values for age 2 and 3 cage-associated *G. morhua* were consistent with the expected low weight and condition of age 2 and 3 *G. morhua* from the local division respectively, and more likely attributable to naturally occurring local variance (i.e., differences in abiotic conditions), and reduced weight and condition patterns of *G. morhua* from the local division when compared to the outside divisions (DFO 2020). Additionally, it is likely that the frequency of interdivisional mixing amongst *G. morhua* from the outside divisions (DFO 2020) made it difficult to compare metrics as distinct divisions, since some individuals were likely not from the division in which they were captured. However, even though comparisons between cage-associated

G. morhua and the local division were mostly comparable, some exceptions were found. For example, age 2 cage-associated *G. morhua* were in lower condition than age 2 *G. morhua* from the local division, whereas age 4 cage-associated *G. morhua* were both longer and heavier than age 4 *G. morhua* from the local division, even though fatty acid analysis suggested mixed relationships between waste feed consumption and length, weight, and condition of cage-associated *G. morhua* age classes (2-4 years old), which highlighted the benefits, or lack thereof, of waste feed consumption to cage-associated *G. morhua*, relative to age class.

LA and ALA, used here and previously in Chapter 2, as biomarkers for waste feed consumption, revealed some paradoxical relationships. For example, given the significant positive relationships between LA / ALA and length and weight of age 2 cage-associated *G. morhua*, one would have expected the increased waste feed consumption would have resulted in longer and heavier age 2 *G. morhua* when compared to those from the local division. However, this was not the case, as the age 2 cage-associated *G. morhua* were of a comparable length and weight to age 2 *G. morhua* from the local division and of lower condition. Further, despite the absence of any significant relationships between LA or ALA and length, weight, and condition, age 4 cage-associated *G. morhua* were both longer and heavier than age 4 *G. morhua* from the local division. Waste feed can provide a predictable energetic subsidy that minimizes foraging time and energy expenditures (Stephens & Krebs 1987, Scales et al. 2015), and consequently has been observed to increase the length, weight, and condition of wild cage-associated fish (Skog et al. 2003, Fernandez-Jover et al. 2007, Dempster et al. 2011). However, the nutritional benefits of

waste feed consumption may only result in increased growth and condition when the cage-associated fish are of a comparable life stage and species to the farmed fish (Masagounder et al. 2016, Hua et al. 2019). Whenever this is not the case, such as in my study (i.e., juvenile *G. morhua* consuming adult *S. salar* feed), it is possible the benefits of farm waste consumption may be reduced or non-existent (Fernandez-Jover & Sanchez-Jerez 2015).

Modern aquaculture feed is formulated to maximize the developmental efficiency of the farmed species (Encarnação 2016), however nutritional requirements vary by species (Molina-Poveda 2016) and change with development (Carter 2015), thus aquafeed may not satisfy nutritional and EFA proportion requirements considered critical for proper marine fish development (Sargent et al. 1999, Glencross 2009) in some species. A lack of nutritious food options for young cage-associated *G. morhua* can result in periods of reduced growth, typically attributed to poor food quality and restrictive feeding regimens as well as size-dependent mortality (reviewed in Ali et al. 2003), which have been followed by a period of accelerated 'compensatory' growth in *G. morhua* once conditions improve (Bélanger et al. 2002).

Compensatory growth has been shown to increase risk tolerance and consequently predation-risk (Álvarez 2011), which may result in an accelerated life-history strategy in young cage-associated *G. morhua* that accept a readily accessible food source trade-off for an elevation in predation risk and mortality rates (Gilliam & Fraser 1987, Abrahams & Dill 1989). However, environmental cues produced by sea cage aquaculture can be misleading and may result in ecological traps for young wild fish (Robertson et al. 2013,

reviewed in Swearer et al. 2021). Fernandez-Jover et al. (2008), suggested that increased anthropogenic activity and pressure around sea cages in the Mediterranean could offset the growth and condition benefits associated with waste feed consumption, and may explain the reduced growth and size of juveniles associated with sea cages (Fernandez-Jover & Sanchez-Jerez 2015), and similar artificial structures (Hallier & Gaertner 2008, Zhou et al. 2018).

Young cage-associated *G. morhua* could therefore provide recruitment to older *G. morhua*, despite increased presence of tertiary predators (Sanchez-Jerez et al. 2008, Arechavala-Lopez et al. 2013, 2015) and predation-risk (Dempster et al. 2002), and conspecific competition (Fernandez-Jover et al. 2009). For the young cage-associated *G. morhua* that survive these stressors (e.g., age 4 *G. morhua*), my results suggest that these individuals may benefit from compensatory growth, which has been shown to increase feed utilization and growth efficiency (Russell & Wootton 1992, reviewed in Abdel-Tawwab et al. 2006, Yengkokpam et al. 2013), assuming age 4 *G. morhua* were also associated with sea cages as juveniles and experienced a similar life history (i.e., waste feed consumption, growth and condition patterns) as age 2 and age 3 cage-associated *G. morhua* from my study. Fernandez-Jover et al. (2007), found increased condition of cage-associated horse mackerel *Trachurus mediterraneus* in the Mediterranean and cage-associated saithe *Pollachius virens* and *G. morhua* in Norway (Fernandez-Jover et al. 2011b) through predominately adult aggregations (Dempster et al. 2002), which experience reduced predation risk (Baird et al. 2019). Therefore, predatory fish may receive an energetic benefit through the consumption of aggregated prey fish (Sanchez-

Jerez et al. 2008, Serra-Llinares et al. 2013, Arechavala-Lopez et al. 2015), including juveniles from more than 20 species (Fernandez-Jover & Sanchez-Jerez 2015), that consume waste feed and can reach abundances in the thousands (Fernandez-Jover et al. 2009, Bagdonas et al. 2012). Additionally, cannibalism comprises a significant dietary input for *G. morhua* (Ciannelli et al. 2007, Puvanendran et al. 2008) and the consumption of escaped farmed juvenile *G. morhua* by wild adult *G. morhua* has been observed in the vicinity of Norwegian sea cages (Serra-Llinares et al. 2013). Therefore, cannibalism may be associated with an increased mortality risk and result in lower condition of cage-associated juveniles but provide an additional energetic benefit to older *G. morhua*. This ontogenetic shift is consistent with Bagdonas et al. (2012), who observed that younger cage-associated *G. morhua* fed directly on waste feed, while older *G. morhua* fed on aggregated prey fish and particularly *P. virens*.

Waste feed consumption has been shown to assimilate into *G. morhua* fatty acid profiles within several months (Olsen et al. 2015), therefore the low LA and ALA concentrations overall in age 3 and age 4 cage-associated *G. morhua* is somewhat surprising, and suggests either insufficient waste feed consumption or poor LA and ALA retention and transfer to higher trophic levels (reviewed in Dalsgaard et al. 2003, White et al. 2019). Therefore, an alternative explanation could be that age 4 *G. morhua*, which demonstrate larger scale habitat connectivity patterns in coastal areas (reviewed in Petitgas et al. 2013), may associate with sea cages opportunistically (Uglem et al. 2008, 2009, Otterå & Skilbrei 2014), and for time frames and / or consumption rates insufficient for LA and ALA assimilation in the liver. Additionally, sea cages can alter the

distribution of wild fish at spatial scales upwards of 82 km^2 (Giannoulaki et al. 2005), and their structural and energetic benefits (reviewed in Callier et al. 2018) may attract a disproportionate number of longer and heavier transient *G. morhua* from outside divisions to sea cages which could explain the longer and heavier age 4 *G. morhua* in my study.

Although the association of wild fish assemblages with sea cages is clear, the results of this study were somewhat paradoxical, and supports evidence that suggests evaluations for costs and benefits of cage association are complex (Bacher et al. 2013). Due to an observed variation in *G. morhua* growth and condition patterns, future research areas should address this variation in relation to life stage and wild fish species associating with sea cages. Identifying older cage-associated *G. morhua* natal grounds could improve our understanding of younger cage-associated *G. morhua* survivorship, recruitment rates and associated life history strategies, and eventually our understanding of species and environmental interactions within these novel environments. Additionally, otolith microchemistry has been used previously to identify wild fish natal grounds (reviewed in Campana 1999, 2005), artificial habitat use (Andronis et al. 2017) and pollutant assimilation (Søndergaard et al. 2015). Therefore, otolith microchemistry may be a logical next step to determining residency timeframes of wild cage-associated fish, relative to life stage and species, and consequently determine whether age 4 cage-associated *G. morhua* in this study were in fact associated with sea cages as younger *G. morhua* (2-3 years). Lastly, rapid environmental change due to cage development can increase stress-linked glucocorticoid concentrations in fish (Sadoul & Geffroy 2019) and suppress growth and condition (Pickering et al. 1991, Sadoul & Vijayan 2016). Therefore,

an analysis of environmental stress to younger cage-associated fish could investigate the role, if any, of environmental change, and particularly predation risk, on the growth and condition of cage-associated wild fish that includes *G. morhua*.

3.5 Conclusion

In conclusion, the significant positive relationships between waste feed consumption (i.e., LA and ALA concentrations) and length and weight in age 2 cage-associated *G. morhua* supported my hypothesis of waste feed consumption in younger cage-associated *G. morhua*; however, the effects of waste feed consumption were paradoxical, as the consumption of waste feed resulted in poorer condition in age 2 *G. morhua* compared to the local reference division (3Ps). Furthermore, accelerated growth was detected in age 4 cage-associated *G. morhua* compared to the local reference division, despite no evidence to support direct or indirect waste feed consumption by age 4 *G. morhua*. Nevertheless, effects of waste feed consumption were not large enough or of sufficient quantities to disrupt growth related patterns across surrounding *G. morhua* populations.

3.6 Tables

Table 3.1 Sample groups, sample sizes (n), and length (cm), weight (g), and condition (Fulton's index, *k*) corrected to a common sampling month ± standard error (SE) of wild Atlantic cod *Gadus morhua* ages 2-4 years old collected from the direct vicinity of sea cages in Pools Cove (Study site) and reference sites removed from aquaculture operations within the local division (3Ps) and outside divisions (3L, 3N, 3O) in Newfoundland, Canada. See Table 3.1S for length, weight, and condition values prior to correction for a common sampling month.

Groups	n	Length	Weight	Condition
Study site	72			
Age 2	42	23.90 ± 0.42	117.39 ± 7.33	0.83 ± 0.02
Age 3	16	35.88 ± 0.77	368.78 ± 23.67	0.78 ± 0.01
Age 4	14	44.57 ± 0.84	745.75 ± 46.05	0.83 ± 0.02
3Ps	329			
Age 2	43	23.79 ± 0.38	145.01 ± 9.23	1.03 ± 0.02
Age 3	142	34.98 ± 0.40	350.41 ± 14.98	0.79 ± 0.01
Age 4	144	40.80 ± 0.44	561.19 ± 29.09	0.81 ± 0.02
3L	546			
Age 2	190	25.00 ± 0.25	142.09 ± 4.90	0.85 ± 0.01
Age 3	182	31.54 ± 0.33	285.68 ± 8.60	0.86 ± 0.01
Age 4	174	40.84 ± 0.33	617.59 ± 15.01	0.88 ± 0.01
3N	245			
Age 2	107	28.46 ± 0.39	214.83 ± 8.63	0.88 ± 0.01
Age 3	64	34.84 ± 0.55	397.17 ± 20.31	0.89 ± 0.01
Age 4	74	44.55 ± 0.57	851.39 ± 36.45	0.92 ± 0.01
3O	162			
Age 2	75	25.90 ± 0.36	158.32 ± 7.34	0.87 ± 0.01
Age 3	62	33.21 ± 0.63	356.23 ± 19.19	0.91 ± 0.01
Age 4	25	42.04 ± 1.07	736.30 ± 69.85	0.93 ± 0.02

Table 3.2 General linear modelling (GLM) results for length (cm), weight (g), and condition (Fulton's index, *k*) for wild Atlantic cod *Gadus morhua* ages 2-4 collected from the direct vicinity of sea cages in Pools Cove (Study site) and reference sites removed from aquaculture operations within the local division (3Ps) and outside divisions (3L, 3N, 3O) in Newfoundland, Canada. Initial models contained group (G), age (A), sex (S) and their interactions (G × A, G × S, and G × A × S).

Study site	Length			Weight			Condition		
vs.	df	t	p	df	T	p	df	t	p
3Ps									
Age 2	4, 452	-0.16	0.87	4, 449	1.79	0.07	4, 449	9.92	< 0.001
Age 3	4, 461	-0.74	0.46	4, 426	-0.50	0.62	4, 426	0.09	0.93
Age 4	4, 426	-2.79	< 0.01	4, 358	-2.55	< 0.05	4, 358	-0.77	0.44
3L									
Age 2	4, 452	1.89	0.06	4, 449	2.06	< 0.05	4, 449	1.61	0.11
Age 3	4, 461	-3.63	< 0.001	4, 426	-2.30	< 0.05	4, 426	3.35	< 0.001
Age 4	4, 426	-2.78	< 0.01	4, 358	-1.85	0.06	4, 358	1.67	0.09
3N									
Age 2	4, 452	7.37	< 0.001	4, 449	7.60	< 0.001	4, 449	3.03	< 0.01
Age 3	4, 461	-0.81	0.42	4, 426	0.73	0.46	4, 426	4.30	< 0.001
Age 4	4, 426	-0.02	0.99	4, 358	1.46	0.15	4, 358	3.14	< 0.01
3O									
Age 2	4, 452	3.05	< 0.01	4, 449	3.02	< 0.01	4, 449	2.28	< 0.05
Age 3	4, 461	-2.08	< 0.05	4, 426	-0.32	0.75	4, 426	5.27	< 0.001
Age 4	4, 426	-1.57	0.12	4, 358	-0.11	0.91	4, 358	2.87	< 0.01

Table 3.3 General linear modelling (GLM) results for length (cm), weight (g), and condition (Fulton's index, *k*) and linoleic acid (LA, 18:2ω6) and α-linolenic acid (ALA, 18:3ω3) proportions (i.e., biomarkers of waste feed consumption) for wild Atlantic cod *Gadus morhua* ages 2-4 collected from the direct vicinity of sea cages ('cage-associated') in Pools Cove, Newfoundland, Canada.

Age	Measurement	Fixed Effects	df	n	t	p
2	Length	LA	1, 24	26	3.05	< 0.01
		ALA	1, 24	26	3.24	< 0.01
	Weight	LA	1, 24	26	3.66	< 0.01
		ALA	1, 24	26	3.95	< 0.001
	Condition	LA	1, 24	26	1.63	0.12
		ALA	1, 24	26	1.66	0.11
3	Length	LA	1, 13	15	0.41	0.69
		ALA	1, 13	15	0.52	0.61
	Weight	LA	1, 13	15	0.23	0.82
		ALA	1, 13	15	0.35	0.74
	Condition	LA	1, 13	15	-0.93	0.37
		ALA	1, 13	15	-0.87	0.40
4	Length	LA	1, 6	7	-0.26	0.80
		ALA	1, 6	7	-0.45	0.67
	Weight	LA	1, 6	7	-0.54	0.61
		ALA	1, 6	7	-0.40	0.70
	Condition	LA	1, 6	7	-0.66	0.54
		ALA	1, 6	7	0.15	0.89

3.7 Figures

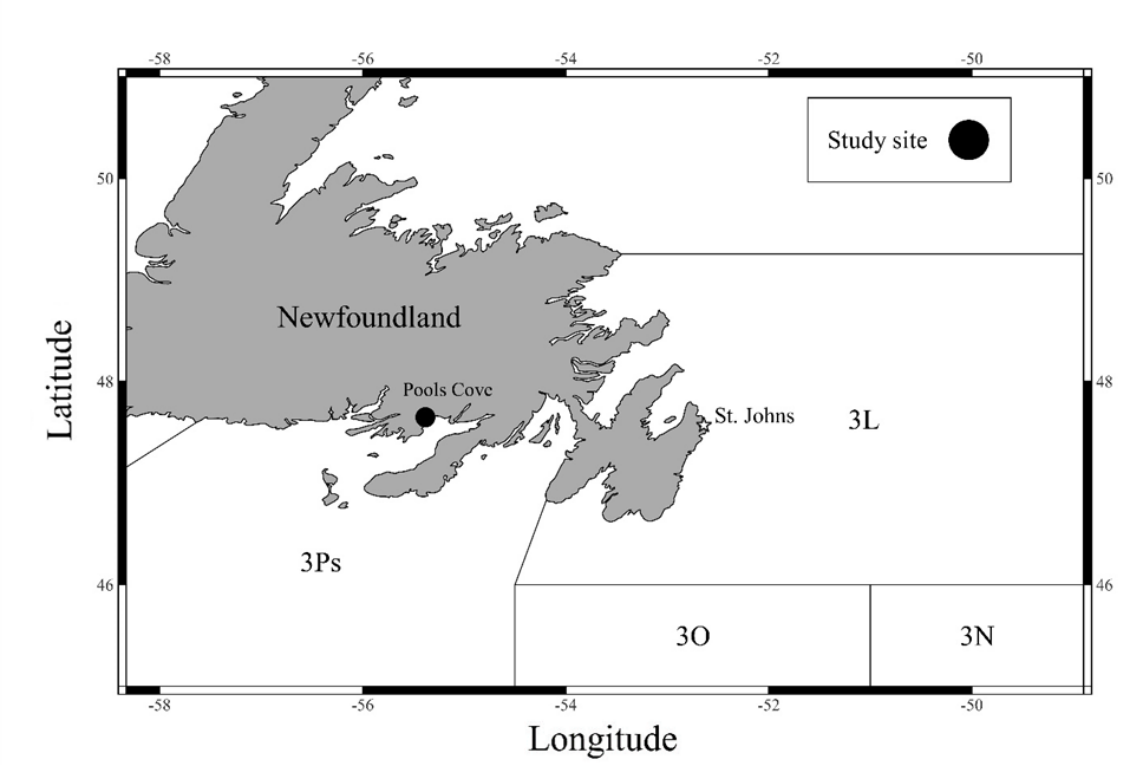

Figure 3.1 Overview of commercial fishing divisions in Newfoundland, Canada used to describe collection sites for the study. Wild cage-associated Atlantic cod *Gadus morhua* were collected in the direct vicinity of sea cages in Pools Cove (Study site) with permission from local aquaculture companies and reference wild *G. morhua* were collected from sites removed from aquaculture operations within the local division (3Ps) and outside divisions (3L, 3N, 3O).

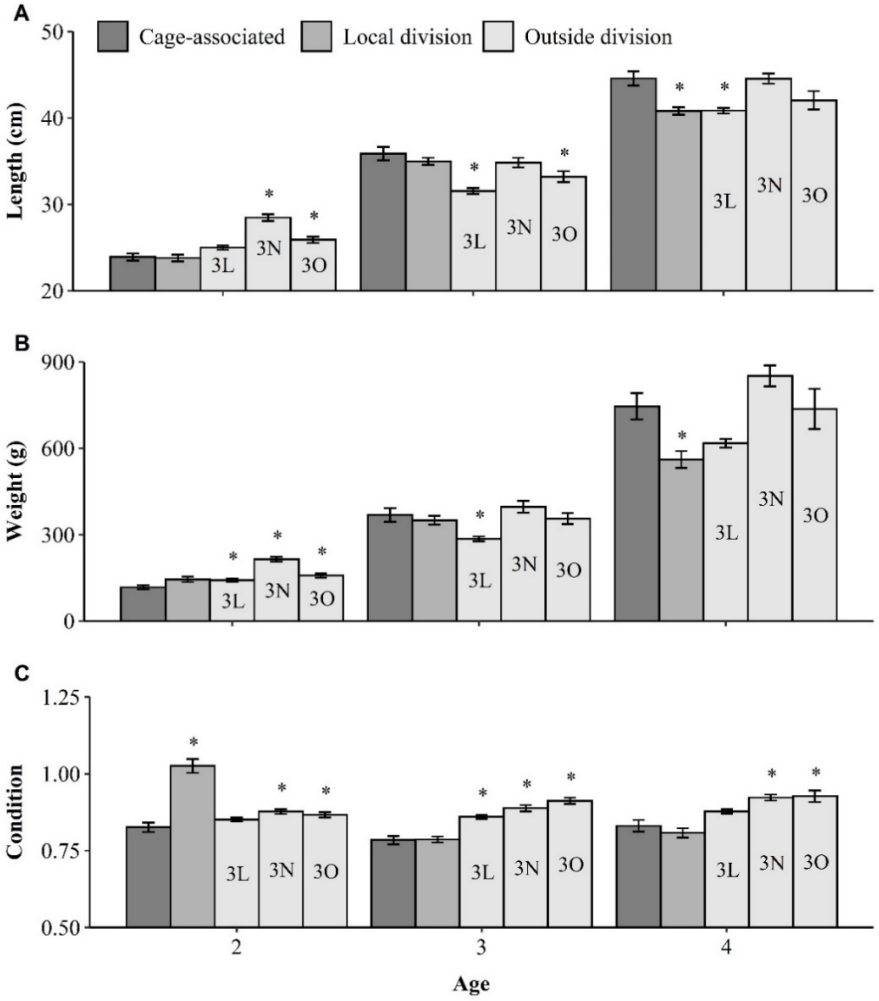

Figure 3.2 Mean (± SE) (A) length (cm), (B) weight (g) and (C) condition (Fulton's index, *k*) values corrected for a common sampling month for wild Atlantic cod *Gadus morhua* ages 2-4 collected in the direct vicinity of sea cages in Pools Cove, Newfoundland, Canada (cage-associated; *darkest grey*) and reference sites removed from aquaculture operations within the local division (3Ps; *lighter-grey*) and outside divisions (3L, 3N, 3O; *lightest grey*). Asterisks denote significant differences ($p < 0.05$) from the cage-associated group.

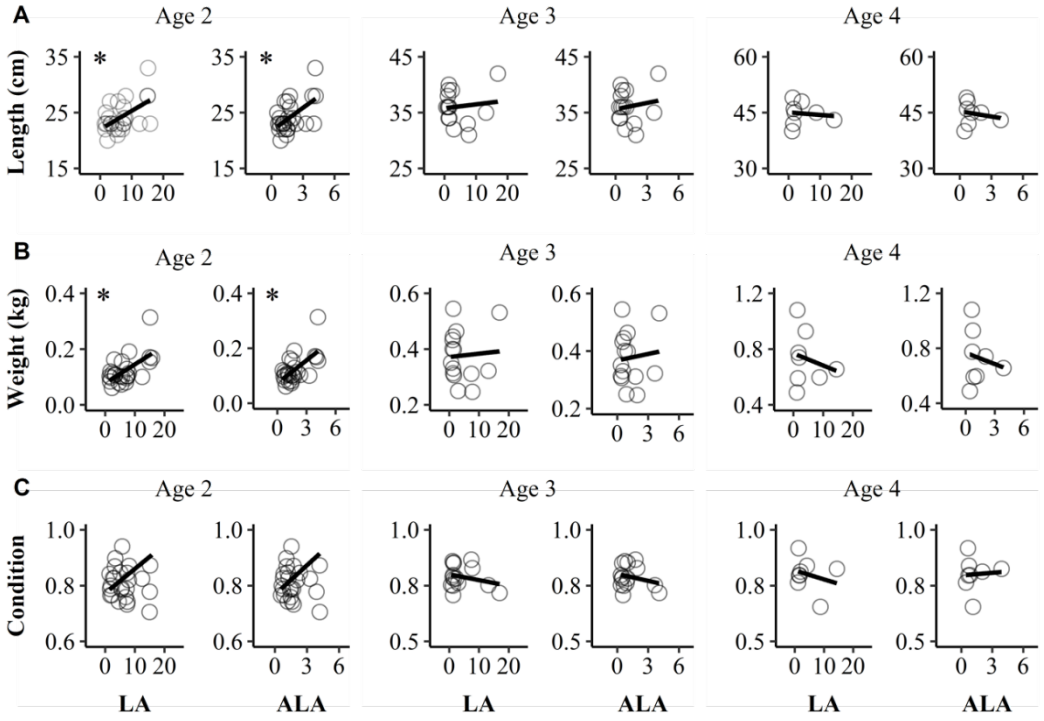

Figure 3.3 Relationships between (A) length (cm), (B) weight (kg) and (C) condition (Fulton's index, *k*) and linoleic acid (LA, 18:2ω6) and α-linolenic acid (ALA, 18:3ω3) concentrations (i.e., biomarkers of waste feed consumption) for wild Atlantic cod *Gadus morhua* ages 2-4 collected in the direct vicinity of sea cages in Pools Cove, Newfoundland, Canada. Asterisks denote significant relationships ($p < 0.05$) between length, weight, and condition and LA / ALA concentrations.

Chapter 4. General Conclusion

The research contained within this thesis determined the short-term sea cage use and dietary habits of Atlantic cod *Gadus morhua* and Atlantic redfish *Sebastes fasciatus* associated with sea cages, and identified non-exclusive underlying mechanisms driving their association with sea cages relative to species and life stage. Additionally, I determined the effects of waste feed consumption on the length, weight, and condition of cage-associated *G. morhua* ages 2-4 years old. In Chapter 2, I successfully discriminated dietary patterns of cage-associated *G. morhua* and *S. fasciatus* using muscle tissue stable isotope values of carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$), and liver proportions of vegetable oil-based fatty acids (VOFAs): oleic acid (OA, 18:1ω9), linoleic acid (LA, 18:2ω6) and α-linolenic acid (ALA, 18:3ω3) and the omega-6 : omega-3 ratio (ω6 : ω3 ratio). Juvenile *G. morhua* (length ≤ 45 cm) $\delta^{13}C$ and $\delta^{15}N$ values and VOFA proportions were indicative of a consistent period of sea cage occupation, and waste feed consumption similar to laboratory *G. morhua* fed an aquafeed diet. Additionally, differences in $\delta^{13}C$ and $\delta^{15}N$ values and proportions of VOFAs were predominately absent in adult *G. morhua* (length ≥ 46 cm) and *S. fasciatus*. Furthermore, Chapter 3 demonstrated the effect of sea cages on the length, weight, and condition of cage-associated *G. morhua* ages 2-4 years old through comparison within the local reference division (3Ps) and outside reference divisions (3L, 3N, 3O). Relationships were also identified between length, weight, and condition and LA / ALA proportions (i.e., biomarkers for waste feed consumption) of cage-associated *G. morhua*, to assess the effects of waste feed consumption among age classes. However, the effects of waste feed

consumption were not large enough to transcend growth and condition patterns among surrounding divisions, though some exceptions were found. For example, age 2 cage-associated *G. morhua* were of lower condition than those from the local reference division, whereas age 4 *G. morhua* were longer and heavier.

Structure availability and a readily available trophic subsidy should be highly attractive to both juvenile and adult fish (see Stephens & Krebs 1987, Scales et al. 2015, reviewed in Callier et al. 2018), however determining the net-benefits of cage association for wild fish can be complex (Bacher et al. 2013), and my results suggest may be dependent on species and life stage. However, the reduced condition of age 2 cage-associated *G. morhua*, in my study, relative to those from the local reference division, is consistent with reduced growth and condition of juvenile fish species associated with sea cages (Fernandez-Jover & Sanchez-Jerez 2015) and similar artificial structures (Hallier & Gaertner 2008). Therefore, young *G. morhua* may use the structural and energetic benefits of sea cages (reviewed in Callier et al. 2018), similar to sea grass or cobblestone systems (Gotceitas & Brown 1993, Lilley & Unsworth 2014), to fulfill nursery requirements (Heck et al. 2003). Accordingly, Fernandez-Jover et al. (2009) observed abundances of juvenile fish in the tens of thousands within the vicinity of sea cages in the Mediterranean, which were not limited by food and demonstrated close site-fidelity to cage structures, presumably to benefit from the available shelter. Therefore, the reduced condition and paradoxical relationships between length and weight and waste feed consumption of age 2 cage-associated *G. morhua*, may be attributed to nutritional variability, as aquafeed formulations are developed to maximize the growth and

development of the farmed species (reviewed in Hua et al. 2019). However, nutritional requirements are variable and dependent on life stage and species (Encarnação 2016, Molina-Poveda 2016), which could limit the physiological benefits of aquafeed consumption to cage-associated wild fish. Further, cage-associated juvenile fish encounter environmental stressors that include increased predation-risk from largely adult and predatory wild fish aggregations (Dempster et al. 2002, Sanchez-Jerez et al. 2008) and large-scale conspecific competition (Fernandez-Jover et al. 2009), which can reduce fish growth and condition (Pickering et al. 1991, Sadoul & Vijayan 2016) and may offset any growth and condition benefits associated with waste feed consumption.

Additionally, an increased length and weight of age 4 cage-associated *G. morhua* was consistent with an increased length, weight, and condition of adult cage-associated wild fish observed elsewhere (Skog et al. 2003, Fernandez-Jover et al. 2007, 2011b). An increased length and weight of age 4 cage-associated *G. morhua* could be attributed to reduced predation risk (Baird et al. 2019), and reduced net-energetic expenditure (MacArthur & Pianka 1966) from the readily available aggregations of prey and farmed fish (Sanchez-Jerez et al. 2008, Fernandez-Jover et al. 2009) around sea cages, including *G. morhua* (Serra-Llinares et al. 2013). Thus, the absence of cage-associated isotopic and fatty acid biomarkers in adult *G. morhua* could be attributed to poor metabolic retention and trophic transfer of LA / ALA proportions (Auel et al. 2002, Dalsgaard et al. 2003), though alternatively in agreement with Sæther et al. (2012), who observed ontogenetic shifts away from direct pellet feeding in adult *G. morhua*. Bagdonas et al. (2012), observed similar behaviour in cage-associated *G. morhua* in Norway, with smaller *G.*

morhua feeding on waste feed and larger *G. morhua* feeding on aggregated prey fish. Therefore, differences in benefits of cage-association by older and younger *G. morhua* suggest additional factors including competition, life stage and predation risk that must also be considered (Fretwell & Lucas 1969, Dahlgren & Eggleston 2000, Craig & Crowder 2002, Bartolino et al. 2011), and could affect the habitat quality and fitness benefits of sea cages to associated wild fish.

Dietary patterns and associated effects suggest that the relative benefits of sea cages should be based on the particular species and life stage, and may not result in universal patterns among fish. Sea cage use patterns of wild fish species can be sporadic and involve site fidelity (Uglem et al. 2008, 2009), and future work should consider the use of otolith microchemistry for the identification of nursery grounds (Campana 2005, Stanley et al. 2016), and determination of residency periods of cage-associated wild fish. Additionally, human-induced rapid environmental change (HIREC) associated with sea cage development can increase glucocorticoid concentrations (Robertson et al. 2013, Sadoul & Geffroy 2019) and decrease the growth and condition of cage-associated fish (Pickering et al. 1991, Sadoul & Vijayan 2016). Continuous or long-term monitoring of sea cage use and dietary patterns, and associated effects for the spatiotemporal variations of wild fish aggregations (Dempster et al. 2002), could provide insight into additional mechanisms that result in wild fish cage-association, which can improve ecosystem management, limit the reduced growth and condition associated with sea cage development (HIREC) and maximize the productivity of these novel ecosystems.

Aquaculture has remained the fastest growing food production sector for decades (Hua et al. 2019), with expansion occurring predominately in coastal sea cages (Lin et al. 2019), which are developed in nearshore habitats that serve as important nurseries to facilitate ontogenetic development for species with complex life cycles (Beck et al. 2001). Additionally, cage-associated wild fish pose implications for recycling farm waste (reviewed in Uglem et al. 2014) and wide-spread effects on local ecosystem structure and health (reviewed in Kalantzi & Karakassis 2006, Cullain et al. 2018). Nevertheless, the potential increases of wild fish abundances, biomass and fisheries production (Dempster et al. 2002, Machias et al. 2006, Uglem et al. 2014) associated with sea cages, could ease the pressure on current wild stocks and act as population sources (Dempster et al. 2011), with substantial economic incentives (FAO 2022). Consequently, these combined effects of sea cage aquaculture on local ecosystems (Dempster et al. 2011) and anthropogenic development (FAO 2022) are of considerable interest, and warrant further study within cage-associated wild fish populations (reviewed in Callier et al. 2018). However, my results similarly support that sea cages provide an energetic subsidy to cage-associated *G. morhua* and can increase length and weight (Skog et al. 2003, Fernandez-Jover et al. 2011a), though benefits were relative to age class, and the identification of consistent site-residency patterns is necessary to further understand the life history of wild fish aggregated within these novel artificial habitats.

In conclusion, the research contained within this thesis is a step towards the identification of the underlying mechanisms present within sea cage aquaculture environments (Sri & Kirubagaran 2015), which result in large-scale spatial distribution changes of wild fish (Giannoulaki et al. 2005), as observed in NL (Goodbrand et al. 2013) and elsewhere (Dempster et al. 2002, Sanchez-Jerez et al. 2008, reviewed in Uglem et al. 2014). Additionally, this research also improves our understanding of the effects of sea cage occupation and the benefits of waste feed consumption on cage-associated *G. morhua* relative to age class, and consequently the non-exclusive physiological costs / benefits of sea cage environments to associated wild fish in NL, Canada. Finally, stable isotopes $\delta^{13}C$ and $\delta^{15}N$, VOFAs (i.e., OA, LA, ALA) and the ω6 : ω3 ratio were successfully used as complementary biomarkers during this thesis to determine sea cage use habits of wild fish, and may possess applicability for use elsewhere in a similar capacity.

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Appendices (*in order of appearance*)

Table 2.1S General linear modelling (GLM) results for proportions (percentage of fatty acid profile) of omega-6 (ω6) fatty acids (FAs), omega-3 (ω3) FAs, arachidonic acid (ARA) and docosahexaenoic acid (DHA) proportions for juvenile and adult Atlantic cod *Gadus morhua* and adult Atlantic redfish *Sebastes fasciatus* collected from sea cage sites and reference sites, and laboratory-reared juvenile *G. morhua* fed an aquafeed diet or squid diet. Initial models contained group (G), length (L), species (S) and their interactions (G × L, G × S, S × L and G × L × S).

Models	Fixed Effects	df	n	F	p
ω6 FAs					
Laboratory *G. morhua*	Group	1,12	14	35.12	< 0.001
Juvenile *G. morhua*	Group	1,57	59	10.09	< 0.01
Adult *G. morhua*	Group	1,21	23	1.25	0.28
S. fasciatus	Group	1,25	27	3.72	0.07
ω3 FAs					
Laboratory *G. morhua*	Group	1,12	14	29.42	< 0.001
Juvenile *G. morhua*	Group	1,57	59	1.55	0.22
Adult *G. morhua*	Group	1,21	23	0.07	0.35
S. fasciatus	Group	1,25	27	20.49	< 0.001
ARA (20:4ω6)					
Laboratory *G. morhua*	Group	1,12	14	4.65	0.05
Juvenile *G. morhua*	Group	2,56	59	0.03	0.87
	Length			7.05	0.01
Adult *G. morhua*	Group	1,21	23	4.17	0.05
S. fasciatus	Group	1,25	27	16.21	< 0.001
DHA (22:6ω3)					
Laboratory *G. morhua*	Group	1,12	14	21.56	< 0.001
Juvenile *G. morhua*	Group	3,55	59	47.84	< 0.001
	Length			0.37	0.55
	G × L			8.44	< 0.01
Adult *G. morhua*	Group	1,21	23	1.97	0.18
S. fasciatus	Group	1,25	27	3.64	0.07

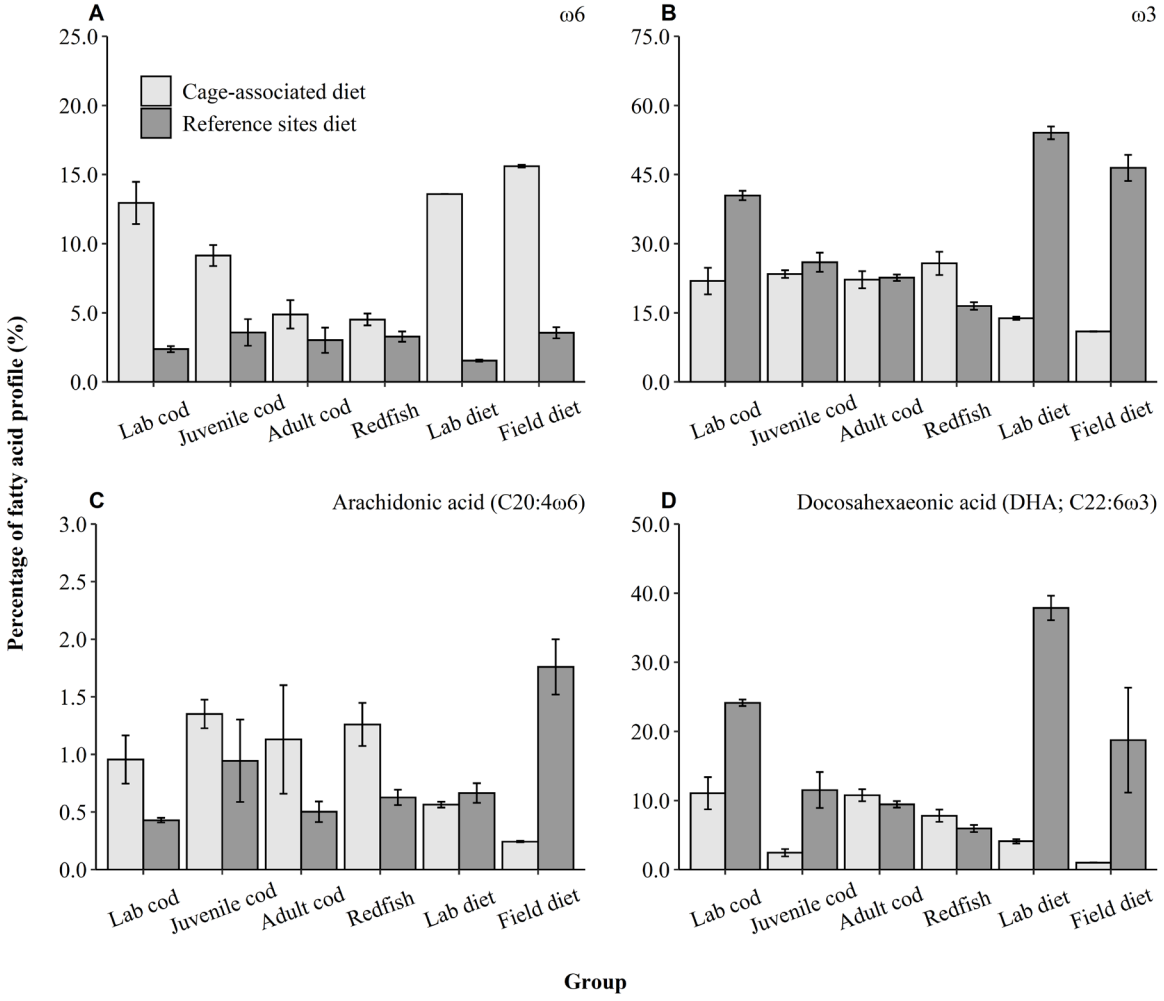

Figure 2.1S Mean (± SE) liver proportions of (A) omega-6 (ω6) FAs, (B) ω3 FAs, (C) arachidonic acid (ARA) and (D) docosahexaenoic acid (DHA) expressed as a percentage of FA profiles for laboratory-reared Atlantic cod *Gadus morhua* fed either an aquafeed diet or squid diet and juvenile *G. morhua*, adult *G. morhua* and Atlantic redfish *Sebastes fasciatus* collected from sea cage sites and reference sites.

Table 3.1S Sample groups, collection season (spring, summer, fall), sample size (n), and mean length (cm), weight (g), and condition (Fulton's index, *k*) values (± SE) of wild Atlantic cod *Gadus morhua* ages 2-4 collected from reference sites removed from aquaculture operations within the local division (3Ps) and outside divisions (3L, 3N, 3O).

Groups	Season	n	Length	Weight	Condition
3Ps		329			
Age 2	Spring	41	22.88 ± 0.38	94.88 ± 6.50	0.76 ± 0.02
	Fall	2	25.00 ± 3.00	190 ± 0.00	0.87 ± 0.00
Age 3	Spring	102	28.25 ± 0.43	183.43 ± 8.32	0.76 ± 0.01
	Summer	4	39.25 ± 0.95	–	–
	Fall	36	39.25 ± 0.81	438.00 ± 89.80	0.83 ± 0.03
Age 4	Spring	67	39.25 ± 0.69	496.25 ± 30.26	0.76 ± 0.01
	Summer	17	40.06 ± 0.97	–	–
	Fall	60	43.45 ± 0.72	667.86 ± 59.51	0.92 ± 0.03
3L		546			
Age 2	Spring	50	22.78 ± 0.33	93.60 ± 4.30	0.77 ± 0.01
	Fall	140	27.41 ± 0.35	193.84 ± 8.53	0.87 ± 0.01
Age 3	Spring	78	29.32 ± 0.49	207.05 ± 10.50	0.77 ± 0.01
	Summer	1	39.00 ± 0.00	470.00 ± 0.00	0.79 ± 0.00
	Fall	103	35.22 ± 0.50	407.86 ± 16.03	0.88 ± 0.01
Age 4	Spring	60	38.83 ± 0.61	493.17 ± 23.83	0.81± 0.01
	Summer	12	39.75 ± 0.84	570.83 ± 33.04	0.90 ± 0.02
	Fall	102	43.25 ± 0.45	769.70 ± 23.23	0.92 ± 0.01
3N		245			
Age 2	Spring	47	25.40 ± 0.49	123.83 ± 7.52	0.71 ± 0.01
	Fall	60	30.50 ± 0.58	275.50 ± 14.88	0.91 ± 0.01
Age 3	Spring	20	30.85 ± 1.09	232.00 ± 27.49	0.73 ± 0.02
	Fall	44	37.50 ± 0.63	507.27 ± 26.63	0.92 ± 0.01
Age 4	Spring	35	41.37 ± 0.80	558.86 ± 39.09	0.75 ± 0.01
	Fall	39	46.67 ± 0.80	1046.41 ± 54.91	0.99 ± 0.01
3O		162			
Age 2	Spring	28	23.18 ± 0.51	102.86 ± 8.34	0.79 ± 0.01
	Summer	1	27.00 ± 0.00	150.00 ± 0.00	0.76 ± 0.00
	Fall	46	26.80 ± 0.50	177.39 ± 10.25	0.87 ± 0.01
Age 3	Spring	46	28.98 ± 0.67	208.04 ± 13.37	0.80 ± 0.01
	Summer	3	36.33 ± 3.28	503.33 ± 114.65	1.01 ± 0.03
	Fall	13	34.23 ±1.20	383.08 ± 42.78	0.91 ± 0.02
Age 4	Spring	13	38.92 ± 1.73	537.69 ± 89.98	0.83 ± 0.03
	Summer	5	44.80 ± 1.39	906.00 ± 115.00	0.98 ± 0.04
	Fall	7	41.86 ± 1.44	728.57 ± 85.54	0.96 ± 0.03

Table 3.2S Slope (m) and intercept (b) values used to correct length (cm) and weight (g) to a common sampling date, and R-squared (R^2) model fitness values, for wild Atlantic cod *Gadus morhua* ages 2-4 years old collected from reference sites within the local division (3Ps) and outside divisions (3L, 3N, 3O) in Newfoundland, Canada.

Reference divisions	Length			Weight		
	m	b	R^2	m	b	R^2
3Ps						
Age 2	0.30	21.36	0.57	12.57	44.47	0.82
Age 3	1.90	19.69	0.92	50.28	-51.82	0.73
Age 4	0.70	35.16	0.59	25.59	356.45	0.43
3L						
Age 2	0.88	17.94	0.99	18.40	-7.77	0.99
Age 3	1.04	23.22	0.80	36.92	-10.73	0.89
Age 4	0.78	34.57	0.82	46.54	241.88	0.96
3N						
Age 2	1.02	20.31	1	30.33	-27.84	1
Age 3	1.33	24.20	1	55.06	-43.27	1
Age 4	1.06	36.08	1	97.51	71.30	1
3O						
Age 2	0.91	18.64	1	18.49	10.42	1
Age 3	1.41	21.92	1	49.40	-38.93	1
Age 4	1.04	33.72	1	66.20	206.68	1

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