# Trophic ecology of Arctic char (*Salvelinus alpinus* L.) in the Cumberland Sound region of the Canadian Arctic

by

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### Abstract

Trophic ecology is a key component in describing patterns of variation between and within populations, particularly in Arctic marine systems wherein climate change is impacting food webs. This thesis investigates the trophic ecology of Arctic char (*Salvelinus alpinus*) in the Cumberland Sound region using a multi-indicator approach. My data show trophic niche differences between resident and anadromous ecotypes and evidence for estuarine feeding by residents. I document a shift in the marine diet of Arctic char from zooplankton to capelin (*Mallotus villosus*) – a novel prey species in this region – that has occurred in less than a decade. Changes in Arctic char growth imply population-level effects of this shift; however, more research is required. Finally, I find lipid effects on  $\delta^{13}$ C and lipid-extraction effects on  $\delta^{15}$ N and  $\delta^{34}$ S for Arctic char muscle tissue. Lipid-correction models did not provide adequate  $\delta^{13}$ C estimates; thus, chemical extraction or ecotype-specific validation of models is recommended.

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### Chapter 1 Literature Review

### 1.1 Trophic Ecology

Understanding the trophic ecology of a species is a key step in describing its role within an ecosystem, as well as patterns of variation both between and within populations. Trophic ecology is often characterized within the framework of the niche concept (Frédérich et al. 2010). A species' niche refers to a multidimensional space consisting of numerous physical and environmental variables (Hutchinson 1957), whereas trophic niches are much narrower and are concerned with niche dimensions pertaining specifically to feeding ecology. Thus, trophic niches are more easily defined and are often characterized in terms of measurable dietary parameters such as prey type (Knudsen et al. 2006).

The dimensions of trophic niches are determined by several factors, including ontogenetic (i.e. developmental) stage (Forseth et al. 1994) and physiological constraints (Portner et al. 2010). Species also tend to partition trophic niches in such a way as to minimize competition (Knudsen et al. 2006). The classic example of trophic niche partitioning is found in Darwin's finches. Congeneric finches minimized trophic niche overlap through specializing on seeds of particular sizes (Grant and Grant 1979). The differentiation of trophic niches can involve divergence of other characteristics related to the partitioning of resource gradients; for example, in the case of Darwin's finches, trophic resource use was related to beak morphology (Grant and Grant 1996). Trophic niche partitioning is also seen at the intraspecific level; for instance, Ehlinger and Wilson (1988) documented differences in the foraging behaviour and morphology of the bluegill sunfish (*Lepomis macrochirus*), identifying two sympatric morphs, one of which was littoral, the other found in pelagic areas of the same lakes. Such trophic niche separations are considered to be a key factor in promoting intraspecific divergence (Jonsson and Jonsson 2001, Knudsen et al. 2006). Thus, identifying intraspecific differences in trophic niches is necessary for understanding adaptive divergence and explaining variation in other traits. More generally, when investigating the role of a species within an ecosystem or understanding population characteristics it is important to identify significant intraspecific differences in trophic niche.

In recent years, an ecosystem-based approach to monitoring changes in natural systems has become increasingly important for ensuring sustainability of natural resources (Gaichas et al. 2010). This is especially applicable to the world's fisheries, where anthropogenic activities are having significant effects on ecosystem structure and function. These effects may be direct, such as exploitation of natural resources (e.g. fishing), or indirect, such as contaminant accumulation and the myriad impacts hypothesized to arise from climate change (Jonsson and Jonsson 2009, Portner and Peck 2010). Climate effects can have significant impacts on marine ecosystems and more specifically on trophic dynamics (van Hal et al. 2010). Climate shifts have been suggested to underpin wide-scale ecosystem changes, including altered species distributions (van Hal et al. 2010) and shifts in productivity at the base of the food web (Brown et al. 2010). For instance, changes in sea surface temperature around Iceland has been cited as a probable factor in changing fish species distribution in the region, including range expansions and contractions, and more frequent occurrences of transient species (Valdimarrson et al. 2012). Trophic ecology seeks to characterize the transfer of energy through an ecosystem, formulated on the basis of feeding interactions (i.e. the

trophic-dynamic concept; Lindeman 1942). In order to understand the anthropogenic impacts and subsequent changes within ecosystems, it is therefore necessary to determine feeding strategies and food web interrelationships.

Describing the trophic niches of species within Arctic systems is particularly relevant, as there is currently much concern over anthropogenic impacts on Arctic ecosystems, including the effects of climate changes and natural resource exploitation (Schofield et al. 2010, Portner et al. 2010). Climate change is having considerable impacts on Arctic environments, leading to altered productivity, changes in species distributions, and shifting food web dynamics, in addition to affecting numerous abiotic properties, such as declining sea ice extent and changes in water temperature and salinity (Schofield et al. 2010, Valdimarrson et al. 2012). These changes can have species-level effects that, in turn, can influence the sustainability of resource exploitation, of which fisheries is a prime example (Brown et al. 2010). As a result, it has become increasingly important to investigate potential changes within a species' trophic niche and the effects of these shifts on populations to allow appropriate management of these systems.

### 1.2 Arctic Char

The high degree of intraspecific variation and the distribution of Arctic char (*Salvelinus alpinus* L.) make it an ideal species for trophic ecology investigations. The Arctic char is the northernmost freshwater fish, its distribution extending throughout the Holarctic region from the northern continental United States to the northernmost Arctic islands (Scott and Crossman 1973). These fish typically occupy post-glacial lakes that are oligotrophic to ultraoligotrophic (Klemetson et al. 2003). The present distribution of Arctic char is thought to reflect the glacial processes of the Pleistocene period (Johnson

1980, Klemetson et al. 2003). Throughout its circumpolar range, the Arctic char exhibits bewildering complexity in almost every aspect of its biology and ecology. Both inter- and intra-population variability has been documented for morphology (Loewen et al. 2009), colouration (Scott and Crossman 1973), feeding strategies (Hindar and Jonsson 1982, Nordeng 1983, Fraser et al. 2008), spawning (Jonsson and Hindar 1982), life history strategies (Loewen et al. 2010) and parasite fauna (Dick and Belosevic 1981), among others. This variability – arguably unparalleled by any other fish species (Klemetson et al. 2003) – has raised many questions concerning the species' taxonomy and ecology and is thus referred to as the "char problem" (Nordeng 1983). Consequently, summarizing the ecology of Arctic char is exceedingly difficult.

### 1.3 Migratory Types

Perhaps the broadest classification encompassing Arctic char variability is migratory type. In general, Arctic char can occur as landlocked, anadromous, and resident migratory types (Scott and Crossman 1973, Nordeng 1983). By definition, landlocked Arctic char occupy lakes lacking a passable connection to the marine environment (i.e. an outlet stream or river), and are generally more southern in distribution (Scott and Crossman 1973), extending as far south as the continental USA (Michaud et al. 2008). However, several landlocked populations have been documented in the high Arctic. For instance, Hammar (2000) described Arctic char populations in landlocked lakes in northern Svalbard, Norway, and Guiguer et al. (2002) documented landlocked Arctic char in northern Ellesmere Island, Canada.

In contrast, the anadromous migratory strategy is generally restricted to populations in northern reaches of the Arctic char distribution (Scott and Crossman 1973), although a

southern migratory population has been identified around Rivière de la Trinité, near the mouth of the St. Lawrence, Canada (Doucett et al. 1999*b*). Anadromous individuals undertake an annual migration to the marine environment to feed, returning to the lake prior to freeze-up for spawning and/or overwintering (Jonsson and Jonsson 1993, Loewen et al. 2009). Thus, these individuals occupy lakes with an outlet stream allowing access to salt water; these connected lake-to-sea habitats will hereafter be referred to open lake systems.

Open lake systems can also contain resident Arctic char (Nordeng 1983). Individuals of the resident ecotype remain in fresh water throughout their entire life cycle, feeding, spawning, and overwintering within the lake despite the presence of a migratory corridor to the sea. Similar to other salmonid species (e.g. brown trout *Salmo trutta*; McCarthy and Waldron 2000), Arctic char populations can be polymorphic with respect to migratory strategy, composed of both anadromous and resident forms (Johnson 1980). These populations are known as partially migratory (Jonsson and Jonsson 1993) and have been documented throughout their distribution (e.g. Loewen et al. 2008, Swanson et al. 2010).

Anadromy is proposed to occur when the benefits of migration outweigh the costs; i.e. when migration offers a fitness advantage (Jonsson and Jonsson 1993). For Arctic char, the main fitness advantage of migration is hypothesized to be access to better feeding opportunities (Gross et al. 1988). The costs of migration may include higher mortality rates in the marine environment, the act of migration itself, and the physiological changes required to adjust to the differences in salinity (Jonsson and Jonsson 1993, Aas-Hanson et al. 2005). Nevertheless, the benefits for anadromous Arctic char can be substantial. Jørgensen et al. (1997) found that anadromous Arctic char can significantly increase their body mass and lipid stores during the marine feeding period, concluding that these benefits offer increased growth for anadromous fish over residents. Rikardsen et al. (2000) simultaneously compared the food intake and growth rates of anadromous and resident Arctic char, and found that anadromous fish were characterized by a much higher, albeit more variable, growth rate, whereas the growth rate of resident Arctic char was low and stable throughout the same time period. From these results, Rikardsen et al. (2000) concluded that foraging in the marine environment offered a fitness benefit for migratory individuals. The life histories of anadromous and resident Arctic char reflect this trade-off. In general, anadromous Arctic char attain a larger size-at-age than residents (Tallman et al. 1996, Loewen et al. 2009). Residents tend to mature earlier and therefore, in accordance with classical life history theory (Stearns 1977), do not live as long as anadromous individuals (Tallman et al. 1996).

Considering that life histories correspond closely to migratory strategy, an understanding of Arctic char trophic ecology is thus crucial to our knowledge of ecotypes. The role of trophic ecology in shaping Arctic char life history characteristics is evident even within an ecotype. Multiple distinct morphs can be present within landlocked populations of Arctic char, and are most commonly identified based on differences in trophic strategy (Jonsson and Jonsson 2001). For instance, in Loch Rannoch, Scotland, Fraser et al. (2008) classified three Arctic char morphs – benthivorous, planktivorous, and piscivorous (i.e. fish-eating) – based on their feeding specializations. In addition, the authors found good correspondence of foraging strategies with life history predictions; the Arctic char with size-limited prey (benthivores and planktivores) were smaller, matured earlier, and had shorter longevity compared to the piscivorous Arctic char. Similar examples of trophic niche partitioning within populations of landlocked Arctic char are abundant in the literature (Hindar and Jonsson 1982, Guiguer et al. 2002, Admundsen et al. 2008, Michaud et al. 2010).

Migratory type and the suite of characteristics (e.g. size-at-maturity, colouration, morphology) that accompany each life history strategy are rooted in the trophic ecology of this species. The differences between anadromous and resident ecotypes reflect the fact that they occupy different trophic niches. However, despite the role trophic ecology plays in the distinction between ecotypes, there have been few comparisons of the trophic niches of sympatric anadromous and resident Arctic char (but see Rikardsen et al. 2000, Swanson et al. 2010). Furthermore, considering the highly variable nature of Arctic char diets, differences in the trophic niche of ecotypes may differ significantly among regions.

In the Cumberland Sound region of Nunavut, anadromous and resident ecotypes coexist within some lake systems, where they have been found to differ in migratory, life history, and morphological characteristics (Loewen et al. 2009, 2010). Trophic niche differences have been inferred, but not directly studied. The trophic ecology of Arctic char is well-studied elsewhere (e.g. Norway; Rikardsen 2000, 2002, 2007; Scotland; Fraser et al. 2008), yet previous research in the Cumberland Sound area is limited (Moore and Moore 1974, Dick and Belosevic 1981), and no previous study in the region has compared the trophic ecology of sympatric ecotypes. In addition, there have been suggestions that resident Arctic char in a Cumberland Sound area lake are utilizing the intertidal habitat (Loewen et al. 2009), suggesting the use of marine trophic resources by residents; this finding warrants further investigation.

### 1.4 Foraging and Diet

Arctic char are known to be highly opportunistic feeders (Moore and Moore 1974). This species has been documented to feed on a wide variety of organisms, from benthos to surface insects to fish. There appear to be three main factors contributing to this variability in Arctic char diets: (1) prey availability, (2) predator size, and (3) competition (Amundsen 1995, Andersson et al. 2005, Eloranta et al. 2010). Risk of predation may also be considered important in determining the trophic resource use of juveniles (Saksgård and Hesthagen 2004, Admundsen and Knudsen 2009). Presently, there is no evidence to suggest that Arctic char diets differ between males and females (Isinguzo 2009).

Of the three main factors identified, prey availability seems to be the predominant factor influencing Arctic char diets, leading to spatial and temporal diet variability both within and among populations (Dempson et al. 2002). One has only to review the lists of prey items exploited by Arctic char to realize that as a species, char are generalists (Moore and Moore 1974). Possibly the best examples of how prey availability affects Arctic char diets at the population level are given by Dempson et al. (2002) and Rikardsen et al. (2007), wherein several years of stomach content data were analyzed. Both studies found that Arctic char diets (specifically large individuals *ca.* > 400 mm) tracked the availability of major forage fish in the regions studied: capelin (*Mallotus villosus*) in Labrador (Dempson et al. (2002) and herring (*Clupea harengus*) in Norway (Rikardsen et al. (2007). Dempson et al. (2002) noted that during years of decreased capelin availability, there were distinct diet shifts from capelin to invertebrates. Similarly, Rikardsen et al. (2007) noted that the representation of herring in the diet of Arctic char declined during years when the documented densities of the area were low.

Given the seasonal variability in abundance and distribution of Arctic aquatic invertebrates (Danks and Oliver 1972, Gu et al. 1999, Zhou et al. 2005), it is not surprising that Arctic char diets vary temporally. Amundsen and Knudsen (2009) found that landlocked Arctic char in Lake Fjellfrøsvatn, Norway, continued to feed during the ice-covered season. In this lake, the summer diet of Arctic char was dominated by surface insects and zooplankton, whereas in winter, they consumed predominantly benthic insect larvae and the amphipod *Gammarus lacustris*. In addition to the seasonal variation, diet was found to vary significantly with Arctic char size, which will be discussed later in this review. Similarly, Amundsen (1995) found that the summer diet of Arctic char in Takvatn, another Norwegian lake, shifted from a diet almost completely dominated by chironomid pupae in June, to one consisting of cladocerans, sticklebacks, *Lymnea* spp., *Gammarus* spp., and tipulidae larvae in October.

As mentioned previously, landlocked Arctic char diets have been well characterized in certain lakes due to the presence of multiple ecotypes, which generally utilize different prey resources. Because lakes vary in their prey compositions, it follows that Arctic char diets will vary depending on specific lake ecology; however, it can be generalized that zooplankton, zoobenthos, and fish are the primary prey components of Arctic char diets in lakes, with insects important in some locations. For instance, Heissenberger et al. (2010) reported that Arctic char from two sub-alpine lakes in Austria fed primarily on zooplankton, but also relied on copepods in one lake and cladocerans in the other. Saksgård and Hesthagen (2004) noted that Arctic char in Lake Atnsjøen, Norway, consumed primarily zooplankton, specifically *Daphnia longispina*, *Bosmina longispina*, *Bythotrephes longimanus*, and *Polyphemus pediculus*. In populations with multiple trophic ecotypes, Arctic char are generally divided into pelagic and benthic forms, feeding on zooplankton and benthos, respectively. In some populations, a cannibalistic feeding strategy also exists. For instance, Guiguer et al. (2002) found that the large-form Arctic char ecotype in Lake Hazen, Canada, were cannibalistic on juveniles.

Arctic char trophic niches shift with fish growth and age (Johnson 1980). Forseth et al. (1994) found that Arctic char in a landlocked lake shifted from a zooplankton to a zoobenthos based diet at ages 3 to 5 years. Similarly, in Fjellfrøsvatn, Norway, Amundsen et al. (2008) documented a shift from zooplankton and chironomid larvae to zoobenthos and surface insects with age. Amundsen et al. (2008) attributed this shift to increases in char size allowing exploitation of larger prey. In some regions, Arctic char shift to piscivory once obtaining certain lengths. The diet of anadromous Arctic char in Norway, characterized by Rikardsen et al. (2007), was found to be comprised primarily of invertebrates at sizes of  $\leq 400$  mm fork length (L<sub>F</sub>), whereas larger fish (> 400 mm L<sub>F</sub>) were predominantly piscivorous. In Labrador, Arctic char less than 150 mm fork length fed on marine zooplankton; they began to feed on fish at fork lengths of 300-349 mm, and their diets were dominated by fish at fork lengths of > 450 mm (Dempson et al. 2002). The development of cannibalism has also been reported to be related to an ontogenetic shift for an Arctic char population from a high Arctic lake in Svalbard (Hammar 2000).

In contrast to the numerous articles concerning the feeding ecology of landlocked Arctic char, especially within Scandinavian lakes (e.g. Amundsen et al. 2008), the marine diet of anadromous Arctic char is relatively poorly documented. As Dempson et al. (2002) pointed out, analysis of the diet of Arctic char feeding in the sea is crucial,

considering that this stage in the life cycle of anadromous populations can have significant fitness benefits. Consistent with the opportunistic nature of Arctic char diets, the diet composition of Labrador, Canada, populations varied widely, even within the same geographic region. Dempson et al. (2002) reported that large (> 300 mm) anadromous Arctic char in Labrador fed primarily on sand lance (*Ammodytes* spp.), capelin, or amphipods, depending on the stock. In a survey of Arctic char stomach contents in Norway, Rikardsen et al. (2000) found that large (>400 mm L<sub>F</sub>) individuals fed on fish such as gadoids (cod), sand lance, and herring. Moore and Moore (1974) sampled Arctic char from several areas within the Cumberland Sound region of Nunavut, Canada: the same region that is the focus for this thesis. Moore and Moore (1974) surveyed prospective food items and identified char stomach contents, finding that the predominant diet items of anadromous Arctic char in the region were planktonic amphipods (*[Para]Themisto libellula* and *Pseudalibrotus glacialis*).

Stable isotope analysis has also been used to demonstrate size-related trophic shifts by comparing the stable isotope ratios of fish of different sizes within a population (Power et al. 2002). In a study of Arctic char from lakes in the Gulf of St. Lawrence region, Power et al. (2002) determined that the nitrogen stable isotope ratio was negatively correlated with fish size. This result was somewhat surprising as it suggested that larger individuals fed at lower trophic levels than smaller individuals. However, the authors noted that increased predator size does not necessarily equate to increased trophic position and suggested that larger fish could have been exploiting prey such as terrestrial insects that became available with larger gape size. Stable isotopes and their use in trophic ecology will be discussed in Section 1.5.

The presence of predators and competitors can alter Arctic char trophic strategies (Saksgård and Hesthagen 2004). Arctic char are known to be a non-aggressive species (Johnson 1980), and it is well-documented that when coexisting with competing fish species, Arctic char occupy a smaller or more specialized trophic niche than if they were the sole species present (Saksgård and Hesthagen 2004, Amundsen and Knudsen 2009). Saksgård and Hesthagen (2004) conducted a long term study of Arctic char and brown trout in Lake Antsjøen, Norway and found that Arctic char specialized on zooplankton whereas brown trout consumed mostly surface insects. Amundsen and Knudsen (2009) provide similar evidence, suggesting that adult brown trout and Arctic char partition their trophic niches such that there is little overlap. The presence of predators seems to be an additional regulator of foraging, particularly in juveniles (Byström et al. 2004). Amundsen and Knudsen (2009) suggested that the presence of brown trout forces juvenile Arctic char to utilize alternate habitat – thus different food items – to reduce predation. Predation pressure may be intraspecific, in the form of cannibalism (as discussed previously), or interspecific.

The trophic variability and flexibility of Arctic char contribute to make Arctic char an ideal study species to understand changes in the environment, as this species can adapt within a relatively short frame (Michaud et al. 2010). Moreover, in the Cumberland Sound area there is reason to suspect a climate change-induced shift in the trophic niche of anadromous Arctic char. In the last decade it has been observed that capelin, a pelagic forage fish, have invaded the Cumberland Sound area (Marcoux et al. 2012). Other northern regions, such as Hudson Bay, have also experienced increases in capelin availability (Gaston et al. 2003). These observations support the suggestion of Huse and

Ellingsen (2008) that capelin would establish new spawning areas in response to predicted climate change scenarios. Capelin populations have historically undergone extensive distribution shifts that have been linked to the species' ability to respond quickly to changes in ocean temperatures, which led Rose (2005) to call capelin a "canary" of the sea with respect to climate changes. Changes in capelin populations can have a cascading effect on ecosystems as they are a key forage species for a wide range of predators, including finfish, marine mammals, and seabirds (Vilhjálmsson 2002, Gaston et al. 2003, Krumsick and Rose 2012, Marcoux et al. 2012). In addition to distribution, there are documented cases of changes in capelin reproductive biology linked to climate conditions (notably water temperature), which include changes in timing and location of spawning, natural mortality, and recruitment (Carscadden et al. 2001, Davoren 2013). Capelin are a short-lived (3 to 6 years), schooling species that are characterized as having high mobility, high energetic needs, and variable recruitment (Carscadden et al. 2001, Vilhjálmsson 2002). In general, capelin mature at 3 to 4 years of age in June, July, or August, and the location of spawning is either intertidal or demersal (Carscadden et al. 2001, Davoren 2013). The feeding ecology of capelin has not been described in the Cumberland Sound region, but capelin diets described from West Greenland and the North Atlantic had similar prey items (O'Driscoll et al. 2001, Hedeholm et al. 2012). Important prey items for capelin in these regions include copepods, hyperiid amphipods, and euphausiids (O'Driscoll et al. 2001, Hedeholm et al. 2012).

Although anadromous Arctic char in the Cumberland Sound region historically relied on invertebrate prey, particularly amphipods (Moore and Moore 1974), preliminary data

show that Arctic char from some areas of Cumberland Sound have incorporated capelin into their diet (R. Tallman, unpubl. data). Capelin is known to be an important forage species for Arctic char in regions where ranges of these two species overlap (Dempson et al. 2002). In Labrador, drastic changes in capelin availability have been linked to shifts in Arctic char diet and proposed changes in Arctic char population characteristics (Dempson et al. 2002, 2008). More specifically, Arctic char growth rates decreased during a period of anomalous climate conditions that was accompanied by significant decreases in capelin availability (Michaud et al. 2010). These considerations led me to question (1) how stable has the trophic niche of anadromous Arctic char in the Cumberland Sound region remained over time, and (2) what effects have possible shifts in diet had on various descriptors of these Arctic char populations (e.g. condition and growth).

Descriptions of trophic niche in fish are generally accomplished using dietary analysis. Fish diets are often studied through analysis of stomach contents (e.g. Moore and Moore 1974, Dempson et al. 2002), which allows the researcher to identify and quantify individual prey items (Cortés 1997). However, stomach contents only provide information on the diet of an individual fish within a short period of time immediately prior to capture. In species with an opportunistic and highly variable feeding strategy – such as Arctic char (Dempson et al. 2002) – a snapshot view of diet may be ineffective in determining overall feeding patterns or trends within a population. Stomach content analysis may also cause biases owing to differential digestion and underrepresentation of soft-bodied organisms, and may pose substantial difficulties in prey identification (Iverson et al. 2004). In addition, logistical constraints may require sampling at times when fish stomachs are empty (e.g. Adams et al. 2003) or using methods that cause

evacuation of gut contents (e.g. Stowasser et al. 2009), precluding the use of stomach content analysis.

To overcome some of the constraints of traditional stomach content analysis, biochemical techniques have been widely adopted for trophic investigations (Post et al. 2002, Iverson et al. 2004). These techniques are based on the premise that certain components of the prey (biomarkers) are incorporated into the tissues of the predator, thus are an indication of the predator's assimilated diet (Iverson et al. 2004). In this way, the pathway of energy flow can be traced through a food web. Biomarkers have the capacity to create a comprehensive picture of diet over the long term, from weeks to months (Bootsma et al. 1996, Wan et al. 2010). Stable isotopes – particularly carbon and nitrogen – and fatty acids are commonly used biomarkers. Analysis of stable isotope ratios, fatty acids, or both have been used in many applications of trophic ecology, including elucidating current and historical food web structure (Hobson and Welch 1992, Vander Zanden et al. 2003), temporal, spatial, and ontogenetic diet variability (Xu et al. 2007, Karnovsky et al. 2008, Eloranta et al. 2010), and migratory patterns (Doucett et al. 1999a, Ciancio et al. 2008). Due to the different types of information provided by stable isotope ratios and fatty acid profiles, the combined use of these biomarkers has allowed a more detailed description of trophic ecology than would be possible using one method alone (Stowasser et al. 2009).

### 1.5 Stable Isotopes

Stable isotopes are now firmly established in the field of trophic ecology and are used for a multitude of applications (Michener and Lajtha 2007). The most commonly used isotopes are those of carbon and nitrogen, which can reveal information on the diet

source and trophic position, respectively, of an organism, due to fractionation along the food web. The sulphur stable isotope ratio ( $\delta^{34}$ S) has also proven to be useful for trophic ecology investigations, particularly for isotopic source differentiation. The stable isotope ratios of carbon ( $\delta^{13}$ C) and sulphur ( $\delta^{34}$ S) show little trophic fractionation, from 0-1 per mil (‰), whereas the nitrogen ( $\delta^{15}$ N) stable isotope ratio increases by 3-4‰ in a stepwise fashion through the links in a food chain (Peterson and Howarth 1987, Vander Zander and Rasmussen 1999). Due to differences in the isotopic ratios of primary producers, researchers are able to identify distinct differences when feeding is based in contrasting environments, such as terrestrial and aquatic (Doucett et al. 1996*a*), benthic and pelagic (Vander Zanden and Rasmussen 1999), and gradients along marine, estuarine, and freshwater habitats (Hesslein et al. 1993, Garcia et al. 2007, Fry and Chumcal 2011).

The main application of stable isotopes in Arctic char biology has been to determine differences in resource use among morphotypes. The bulk of the research compares landlocked morphs, and interesting discoveries have been made concerning trophic resource use, which have complemented stomach content analyses (Adams et al. 2003). For instance, Adams et al. (2003) were able to define different trophic niches for a size-polymorphic Arctic char population in Loch Tay, Scotland. In this lake, mean carbon and nitrogen stable isotopes of Arctic char were significantly different between small and large mature individuals, which the authors attributed to partitioning of foraging niches. Similarly, Guiguer et al. (2002) corroborated trophic differences between morphotypes by analysis of C and N isotopes, determining that the large morph was <sup>13</sup>C-depleted and <sup>15</sup>N-enriched compared to the small morph. These results supported stomach content analysis indicating that large form individuals tended towards a more piscivorous feeding

strategy. It should be noted that stable isotope analysis may not always agree precisely with the results of stomach content analysis. For instance, Power et al. (2002) found that the stable nitrogen signature of Arctic char indicated an ontogenetic shift to a lower trophic level with increased size, an effect that was not apparent with stomach contents alone.

When assessing diet with stable isotopes, the isotopic turnover rate is an important consideration, especially when assessing diet change (Sakano et al. 2005). In a study of Atlantic salmon (*Salmo salar*), Trueman et al. (2005) found that stable isotope turnover in white muscle occurred at a rate of 20-40% per month; although not surprisingly, isotopic turnover rate was related to growth rate. The authors stated that fast-growing fish would quickly (i.e. within a few months) reach tissue-diet isotopic equilibrium (Trueman et al. 2005).

Recently, stable isotopes have been used in determining salmonid migratory strategies (McCarthy and Waldron 2000). The use of stable isotopes for identification of migratory ecotype in partially anadromous salmonid populations was proposed by Doucett et al. (1999*a*), who suggested that differences in the carbon, nitrogen, and sulphur isotopic ratios of adult brook trout reflected their migratory strategy. Because anadromous and resident Arctic char utilize trophic resources based in different environments (i.e. freshwater and marine), and the stable isotope ratios of primary prey in these two environments should be different (Garcia et al. 2007), anadromous and resident fish should therefore exhibit different isotopic signals. Recent work by Ciancio et al. (2008) has provided evidence of this in Patagonian riverine populations of rainbow trout (*Oncorhynchus mykiss*) and brown trout. The authors compared  $\delta^{15}$ N and  $\delta^{13}$ C of

ecotypes from several river systems that had previously been identified using other methods. Ciancio et al. (2008) reported anadromous fish had higher  $\delta^{15}$ N and  $\delta^{13}$ C (15.2 ± 1.0‰ and -19.2 ± 1.3‰, respectively; mean ± standard deviation) compared to the  $\delta^{15}$ N and  $\delta^{13}$ C of residents (8.8 ± 1.1‰ and -23.2 ± 2.5‰, respectively), regardless of variation due to species, capture location, fork length, and fasting. A similar trend was found in the isotope ratios of partially migratory salmonids in the western Canadian Arctic, where mean  $\delta^{15}$ N and  $\delta^{13}$ C of anadromous lake trout (*Salvelinus namaycush*; 16.4 and -22.3‰, respectively) were higher than those of residents (12.8 and -26.21‰, respectively) (Swanson et al. 2010). In general, anadromous fish have higher  $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S, which reflect feeding in different environments (e.g. freshwater versus marine) and feeding at higher trophic levels (for  $\delta^{15}$ N).

The stable isotope profiles of partially migratory populations of Arctic char have been characterized by Swanson et al. (2010) and Doucett et al. (1999*b*). Doucett et al. (1999*b*) performed stable isotope analysis on Arctic char from a river system in Québec, and suggested that those individuals with higher mean (± standard deviation) stable isotope ratios ( $\delta^{15}N = 12.3 \pm 0.5\%$ ;  $\delta^{13}C - 22.1 \pm 1.3\%$ ) were anadromous, whereas those with lower ratios ( $\delta^{15}N = 8.9 \pm 0.4\%$ ;  $\delta^{13}C - 29.2 \pm 1.1\%$ ) were non-migratory. Notwithstanding these few studies there have been few previous applications of isotopic tools and no studies addressing the trophic ecology of partially anadromous Arctic char populations within the eastern Canadian Arctic.

Stable isotopes have also been used successfully to document long-term diet shifts and ecosystem change (e.g. Hebert et al. 2002). Hobson et al. (2004) identified stable isotope ratios of carbon and nitrogen from archived seal teeth, which enabled them to observe patterns that were attributed to changes in foraging location, food web structure, and oceanographic conditions. In freshwater systems, this technique has been used to document drastic shifts in fish diet in response to species invasions (Eagles-Smith et al. 2008). In this thesis, population-specific stable isotope ratios are compared over time to investigate potential long-term temporal trends in anadromous Arctic char diets during the period in which the invasive marine forage fish – capelin – were becoming available.

### 1.6 Fatty Acids

The fatty acid composition of Arctic char in relation to their diets has historically been studied for aquaculture and the quest for diet formulations providing maximum survival, production, and growth of cultured species (Dalsgaard et al. 2003). In recent years, fatty acids have emerged as an important tool in ecology for understanding trophic interactions, food web structure, and energy flow through ecosystems (Iverson et al. 2004, Elsdon 2010). This is especially true in marine systems, where fatty acids are both abundant and diverse (Iverson et al. 2004). Researchers reasoned that if fatty acids were incorporated into their tissues with little modification through the food web, the fatty acid profile – or signature (Iverson 1993) – of predators should approximate that of their prey.

The use of fatty acids as indicators of diet has been well established in higher trophic level marine organisms, namely seabirds and marine mammals (e.g. Karnovsky et al. 2008, Loseto et al. 2009). As previously stated, there has been considerable research in the field of fish lipids and nutrition, mostly due to the aquaculture industry. However, the application of fatty acid analysis as an indicator of fish diet in natural systems has received little attention until recently (e.g. Stowasser et al. 2009, Daly et al. 2010, Wan et al. 2010).

In fish, fatty acids represent the main source of metabolic energy for growth and development (Sargent et al. 1999), which includes reproduction and migration (Tocher 2003). Fatty acids comprise the bulk of lipid content, present in triplet within triacylglycerides, each esterified to an alcohol group of glycerol (Tocher 2003). Tocher (2003) provides a fairly comprehensive review of the general fatty acid composition of fish. Briefly, the carbon chains of fatty acids present in fish generally range from 12 to 24 carbons, with varying degrees of saturation (i.e. the number of double bonds along the carbon chain). Fatty acids with no double bonds are known as saturated fatty acids (SFA) and are represented primarily by 16:0 and 18:0. Monounsaturated fatty acids (MUFA) have only one double bond, and the MUFA most often represented are 18:1n-9 and 16:1n-7, but also important in fish are 20:1n-0 and 22:1n-11 (Tocher 2003). Fatty acids with more than one double bond are referred to as polyunsaturated fatty acids (PUFA). PUFA can be highly variable, especially in marine systems (Iverson et al. 2004), but in general, the PUFA 22:6n-3 (docosahexaenoic acid, DHA), 20:5n-3 (eicosapentaenoic acid, EPA), 20:4n-6 (arachidonic acid, ARA), and 18:2n-6 (linoleic acid; LA) are especially important for fish growth and survival (Sargent et al. 1999, Dalsgaard et al. 2003). PUFA, and particularly DHA, EPA, and ARA, have generated the most interest in trophic studies, because vertebrates are not able to synthesize them *de novo* (Tocher 2003).

It is generally accepted that the fatty acid composition of fish reflects their dietary intake (Dalsgaard et al. 2003). This has been demonstrated experimentally and inferred from studies of natural systems (St. John and Lund 1996, Elsdon 2010). In a seminal paper using fatty acids as diet tracers, St. John and Lund (1996) first validated their

methods by rearing Atlantic cod (Gadus morhua) larvae on a controlled diet of copepods that had been fed one of two phytoplankton species. They found that in as little as eight days, the fatty acid composition of the larvae began to reflect that of their diet; subsequently, the authors used these lipid biomarkers to identify the resource base for cod in wild populations. Elsdon (2010) provided further evidence by experimentally altering the diets of juvenile black bream (Acanthopagrusbutcheri), an estuarine fish, and assessing the changes in fatty acid compositions. This study found that fatty acid analysis could successfully identify diet shifts, and that PUFA (especially LA and DHA) were the most useful indicators of diet. The results of Daly et al. (2010) provide evidence for the use of fatty acids as diet indicators in salmonids. This field study found that the fatty acid composition of juvenile Chinook (Oncorhynchus tshawytscha) and coho (O. kisutch) salmon changed significantly upon switching to a marine feeding strategy, which included significant increases in essential PUFA (particularly DHA), and an alignment of predator fatty acid profiles with those of prey (Daly et al. 2010). Results of an experimental feeding study with coho salmon and rainbow trout indicate that dietary fatty acids are rapidly incorporated into muscle tissue, with maximum concentrations of specific fatty acids occurring in as little as two weeks (Skonberg et al. 1994). This turnover rate is somewhat shorter than is observed for stable isotopes (see Section 1.5).

Caution must be exercised when interpreting the fatty acid compositions of fish for two main reasons, the potential for (1) fatty acid modification and (2) preferential fatty acid retention (Henderson and Tocher 1987, Heissenberger et al. 2010). Although fish are unable to produce LA and  $\alpha$ -linolenic acid (ALA 18:3n-3), it has been suggested that most freshwater fish, including Arctic char, are able to modify these dietary fatty acids into DHA, EPA, and AA in order to meet their physiological requirements (Henderson and Tocher 1987). In contrast, marine fish, and even freshwater fish in the marine environment, are suggested to obtain these essential fatty acids solely from their diets (Tocher 2003). Furthermore, some fish may preferentially retain certain fatty acids (Heissenberger et al. 2010). These authors suggested that several species of salmonids (including Arctic char) are capable of preferentially retaining DHA, based on the observation that the fish DHA levels were significantly higher than dietary levels of this fatty acid.

When studying fatty acid profiles in terms of trophic ecology, potentially confounding biotic and abiotic factors must be considered, as discussed previously for stable isotope analysis. In fish, fatty acid composition may be affected by physiological state, reproductive status, and ontogenetic stage (Dalsgaard et al. 2003). Salmonid migrations between fresh water and salt water have been demonstrated to be accompanied by changes in total lipid content and composition of lipid classes (Jobling et al. 1998). However, from an experimental study with aquaculture Arctic char, Bystriansky et al. (2007) found that changes in salinity did not seem to affect lipid metabolism or plasma fatty acid content. Indeed, the lipid changes observed by Jobling et al. (1998) were attributed to seasonal variation in anadromous Arctic char lipid content reflecting summer feeding opportunities and reproductive status. Given that diet can vary seasonally and that diet and fatty acid composition are interrelated, as discussed previously, it follows that seasonal variations in fatty acid composition exist. Seasonal variation in fatty acid composition has been revealed in deep sea fishes (Stowasser et al. 2009) and salmonids (*Oncorhynchus* spp., Daly et al. 2010). Water temperature does not

seem to have an effect on fatty acid composition; this was tested by Elsdon (2010) on the black bream (*Acanthopagrus butcheri*), an estuarine fish species.

Fatty acid composition differs among tissue types, which is likely related to differential rates of tissue turnover (MacNeil et al. 2006), similar to the difference in stable isotope ratios as previously discussed. For instance, Stowasser et al. (2009) demonstrated distinct differences between the fatty acid compositions of liver and muscle tissue of deep sea fishes. The authors caution that fatty acid composition of the muscle tissue may represent the physiology of the tissue itself, and may not provide an accurate representation of diet, at least in comparison to the liver. However, when choosing a tissue for lipid analysis, the location of lipid storage for the species in question must be considered. In the deep sea fish investigated by Stowasser et al. (2009), liver may be the major storage site for lipids, whereas in Arctic char, skeletal muscle is the second largest storage depot for lipids (35-40%), surpassed only by the carcass (i.e. head, fins, skin, and skeleton at 50%) (Jobling et al. 1998). In Arctic char, muscle fatty acid composition would likely be a better descriptor of diet, because liver is a relatively minor lipid storage site.

In summary, while there are numerous factors to consider when attempting to link fatty acid composition and diet, fatty acid analysis is a tool that can be used successfully as a general descriptor of fish diet. Using these techniques and considerations, this study compares the fatty acid compositions of sympatric anadromous and resident Arctic char, and the results were interpreted alongside stable isotope analysis to describe trophic differences within two partially anadromous populations.
## **1.7** Fisheries Management

Arctic char is a traditional diet item for indigenous peoples in the Canadian Arctic, and in the present day, the subsistence harvest of Arctic char by Inuit is still represented in their diet (Mead et al. 2010). In addition, some Arctic communities, such as the hamlets of Pangnirtung and Cambridge Bay, are now economically invested in commercial Arctic char fisheries, equipped with fish processing infrastructure (Rompkey and Patterson 2010). Considering the increasing economic reliance on these fisheries, steps need to be taken to ensure management practices promote the health of Arctic char populations. These should include prioritizing research that investigates factors that have potential impacts on Arctic char populations, including trophic ecology (Brown et al. 2010). Ecosystem-based management is fast becoming the goal for fisheries worldwide, of which modeling of food webs is an important component (Gaichas et al. 2010). Providing basic ecological information on Arctic char trophic strategies will allow assessment of other contributing factors, including the effects of climate change and shifts in species distributions, allowing fisheries managers to make informed decisions.

## 1.8 Thesis Objective and Hypotheses

This thesis adopts a multi-indicator approach with the objective of investigating the trophic ecology of Arctic char in the Canadian Arctic, employing stomach content, stable isotope, and fatty acid analyses. To facilitate comparisons between ecotypes, among years, and within methods, the thesis has been arranged into three main chapters.

Chapter 2, entitled "Multi-indicator evidence for habitat use and trophic strategy segregation of two sympatric forms of Arctic char from the Cumberland Sound region of Nunavut, Canada", compares the trophic niches of sympatric anadromous and resident

ecotypes. Based on previous research suggesting differences in habitat use between these ecotypes, I hypothesized that anadromous and resident Arctic char occupy significantly different trophic niches. Accordingly, I expected significantly different stable isotope ratios and fatty acid signatures between the ecotypes.

Chapter 3 of this thesis – "Evidence for a trophic shift in Arctic char from the Cumberland Sound region" – examines the temporal trends in the trophic niche of anadromous Arctic char over the past decade. From recent reports of capelin in the Cumberland Sound area, I hypothesized that the trophic niche of anadromous Arctic char has changed over time with the availability of a novel pelagic prey item. I expected that this shift would be reflected in the stable isotope profiles of these Arctic char populations, and that there would be corresponding changes in population characteristics (growth and condition).

Chapter 4 is entitled "The influences of lipids and lipid extraction on carbon, nitrogen, and sulphur stable isotope signatures in a partially migratory Arctic fish species, the Arctic char". This chapter delves into particulars of the methodology employed in Chapter 2 (Section 2.2.4.1). This chapter addressed two main objectives: (1) to determine how lipid extraction affects the stable isotope signatures of Arctic char, and (2) to assess if a lipid-correction model can be applied to this species.

# Chapter 2 Multi-indicator evidence for habitat use and trophic strategy segregation of two sympatric forms of Arctic char from the Cumberland Sound region of Nunavut, Canada

# Abstract

Populations of Arctic char in the Canadian Arctic commonly consist of resident (i.e. non-migratory) and anadromous ecotypes, which were hypothesized to occupy significantly different trophic niches. Arctic char were collected from two study sites (Qasigiyat and Iqalugaarjuit) in the Cumberland Sound region in September 2004 and 2011. Muscle tissue was analyzed for carbon, nitrogen, and sulphur stable isotope ratios  $(\delta^{13}C, \delta^{15}N, \text{and } \delta^{34}S, \text{respectively})$  and for fatty acid composition. Results confirmed that anadromous and resident Arctic char occupied different trophic niches. Resident Arctic char had lower  $\delta^{13}$ C and  $\delta^{34}$ S, indicative of a freshwater feeding, compared to anadromous individuals, for which  $\delta^{13}$ C and  $\delta^{34}$ S indicated marine foraging. The significantly lower  $\delta^{15}$ N of residents relative to anadromous fish suggests the ecotypes feed at different trophic levels, although in the absence of baseline data, absolute differences could not be concluded. Significantly wider  $\delta^{13}$ C and  $\delta^{15}$ N ranges in residents implied a broader trophic niche or a wider range in baseline prey isotope values. Principal component analysis of fatty acid profiles revealed that differences between the ecotypes were mainly due to the PUFA C20:4n6 (ARA), C22:6n3 (DHA), and C18:2n6 (LIN), and SFA (C16:0 and C18:0), which were in higher proportions in resident fish, and MUFA (C20:1 and C22:1) that were in higher proportions in anadromous fish. The MUFA in anadromous Arctic char may be indicative of the importance of copepods in the Cumberland Sound food web. Results also provide further evidence for resident use of the estuarine environment in Qasigiyat. Immature Arctic char appear to occupy a

different trophic niche than both resident and anadromous fish within Iqalugaarjuit, but this relationship is less clear in Qasigiyat. This study provides the first documentation of the fatty acid differences between sympatric anadromous and resident ecotypes and gives evidence for trophic niche segregation of ecotypes within a partially anadromous population.

# 2.1 Introduction

The ecology of the Arctic char is extremely diverse throughout its circumpolar range (Scott and Crossman 1973), including variation in morphology, feeding, and habitat selection (Johnson 1980, Klemetson et al. 2003). In the Canadian Arctic, this freshwater salmonid occupies postglacial lakes, and where these lakes retain a connection to the marine environment (hereafter referred to as open lake systems), Arctic char populations commonly consist of two ecotypes: anadromous and resident (Johnson 1980, Jonsson and Jonsson 1993). Anadromous individuals are those that migrate annually to salt water for the summer months and return to fresh water in the fall for overwintering and/or spawning, whereas residents remain in fresh water year round. These ecotypes possess different life history strategies, and can sometimes be differentiated by their morphology, colouration, and size (Loewen et al. 2009, 2010). Differences in trophic ecology between the ecotypes may be one of the key drivers of divergence; Gross et al. (1988) suggested that a major benefit of migration is access to better feeding opportunities in the sea.

Previous work in the Cumberland Sound region by Loewen (2008) led to the proposal that resident fish occupy a different trophic niche than anadromous individuals, which was interpreted from extensive life history, morphology, and otolith strontium analyses. These fish were captured from two systems, which are the focus of this paper:

Qasigiyat (PG015) and Iqalugaarjuit (PG082). Furthermore, Loewen et al. (2009) presented evidence for estuarine habitat use by resident individuals at Qasigiyat. This strategy may be facilitated by the lake's physiography, which may allow salt water inundation of the freshwater lake during certain times in the tidal cycle (Loewen 2008). Given these findings, it is possible that resident Arctic char in this system utilize marine trophic resources.

Stable isotopes are a useful tool for comparing feeding and habitat use strategies, especially when comparing marine and freshwater systems, because these habitat types often have distinctive isotopic profiles (Fry and Sherr 1984). In one of the first articles to compare resident and anadromous fish using stable isotopes, Hesslein et al. (1993) found that the isotope ratios of lake whitefish (*Coregonus clupeaformis*) were different from all potential food sources in the freshwater lake, indicating a marine-based feeding strategy. Marine-freshwater differentiation reflects the carbon and nitrogen isotopes at the base of the food webs in marine and freshwater environments (Fry and Sherr 1984, Montoya 2007). Sulphur isotopes have also been used successfully to distinguish between marine and freshwater diet and habitat use, as marine  $\delta^{34}$ S signatures tend to be lower than freshwater  $\delta^{34}$ S signatures (Peterson and Howarth 1987, Godbout et al. 2010). Thus, once an ecotype is known, differences in feeding strategies can be compared quantitatively using stable isotopes, and in the future, ecotype affinity of an individual may be based solely on stable isotope data. In contrast to stable isotope profiles, the differences in fatty acid signature between migratory ecotypes have received little, if any, attention. Yet anadromous Arctic char lipid composition is well studied due to the quest for determining the ideal diet for raising productive, healthy individuals in aquaculture (Sargent et al.

1999, Pickova et al. 2007).

Heissenberger et al. (2010) explored the fatty acid composition of Arctic char and other freshwater salmonids in Austria, and examined the relationship between fatty acids and trophic position. The study populations were from two landlocked lakes and two aquaculture operations. The authors found that aquaculture Arctic char had significantly higher polyunsaturated fatty acid (PUFA) concentrations ( $30.6 \pm 11.9 \text{ mg/g}$  dry weight) than lake-dwelling Arctic char (17.5  $\pm$  4.8 mg/g DW). However, the contributions of certain biologically-significant PUFAs were variable: Arctic char in lakes had significantly lower concentrations of docosahexaenoic acid (DHA; 22:6n-3) and higher concentrations of arachidonic acid (ARA; 20:4n-6) than those from aquaculture, whereas there was no difference in  $\alpha$ -linolenic acid (ALA; 18:3n - 3), linoleic acid (LA; 18:2n-6), and eicosapentaenoic acid (EPA; 20:5n-3). The fatty acid found in the highest quantity in the muscle of these Arctic char was DHA, followed by EPA (Heissenberger et al. 2010). Heissenberger et al. (2010) went further to compare these values with the fatty acids of the hypothesized diet, finding that the FA concentrations of prey items were poorly reflected in Arctic char. They suggest that some fatty acids (e.g. DHA) are subject to preferential retention in these fish. Furthermore, fatty acid concentrations did not correlate with  $\delta^{15}$ N; from this, the authors propose that there is no direct relationship between fatty acid concentration and trophic position. However, I would argue that fatty acid composition is a more useful indicator of diet than concentration, as it takes into account the suite of variables (fatty acids) that can be used to compare predator with prey instead of focusing on one fatty acid or fatty acid group. Heissenberger et al. (2010) also did not provide a robust analysis of the data, as they relied solely on univariate

techniques, treating each variable (i.e. individual fatty acids or fatty acid categories) separately instead of analysing the overall fatty acid compositions with multivariate techniques, as is the norm in other studies of fatty acids as indicators of diet (e.g. Daly et al. 2010, Wan et al. 2010).

Previous work on the fatty acid composition of Arctic char has shown that the predominant essential fatty acids in Arctic char are DHA, EPA, and ARA (Pickova et al. 2007, Heissenberger et al. 2010). Thus, I expected to find these fatty acids in the highest proportions. It is now known that some salmonids (including rainbow trout and Atlantic salmon), have the capacity to synthesize DHA and EPA from ALA (Sargent et al. 1999). This mechanism is also hypothesized to exist in Arctic char (Tocher 2003). In addition, DHA seems to be preferentially retained by freshwater salmonids (Heissenberger et al. 2010). Therefore, DHA and EPA may not differ significantly between anadromous and resident ecotypes. However, Daly et al. (2010) found that DHA was the most important fatty acid differentiating between juvenile salmon upon first arrival in the marine environment and those that had fed on marine resources for at least a month.

This study investigates the trophic niches of resident, anadromous, and immature Arctic char in the Cumberland Sound region of the Canadian Arctic using multiple biological indicators: stable isotopes and fatty acids. One of the objectives was to validate the use of stable isotopes for confirming the identity of resident and anadromous individuals prior to assessing trophic niche, which was done by conducting stable isotope analysis on specimens that had previously been identified to ecotype using morphology and otolith strontium profiles (Loewen 2008). The technique was then applied to newly collected samples. The fatty acid profiles of fish muscle tissue were used to provide

additional data for ecotype comparisons. Comparisons were made between ecotypes and between study sites, specifically testing two hypotheses: (1) the anadromous and resident ecotypes have different stable isotope and fatty acid signatures; (2) the differences between ecotypes within the Qasigiyat study site are less pronounced than those from Iqalugaarjuit, given the possibility of resident estuarine feeding.

# 2.2 Materials and Methods

## 2.2.1 Study Sites

Cumberland Sound is an Arctic extension of the North Atlantic, extending northwest from Davis Strait and bordered by the fiords of southeast Baffin Island (Fig. 1). According to the Canadian Hydrographic Service, the marine environment in this region is characterized by extreme tides, with tidal cycles exceeding 7.2 m above and 0.3 m below chart datum (CHS 2011). This study focused on two lake systems featuring freshwater lake basins connected to the marine environment by streams passable for Arctic char: Qasigiyat (PG015), and Iqalugaarjuit (PG082).



**Figure 1.** Location of Iqalugaarjuit (PG082) and Qasigiyat (PG015) study sites in the Cumberland Sound region, showing the community of Pangnirtung, Nunavut. Inset map shows the location of Cumberland Sound along southeast Baffin Island in the Canadian Arctic.

Qasigiyat (64°62'N, 66°31'W) drains into Ptarmigan Fiord near the mouth of Cumberland Sound (Fig. 1). Details of the system's characteristics are given by Loewen (2008) and are summarized here. Only the lower lake basin of the system has been surveyed, as fish passage to the upper lake basin is blocked by large boulders from an historical rock fall (Fig. 2). The lower lake basin reaches a maximum depth of approximately 21 m with steep, fiord-like edges. The lake is in close proximity to the marine environment, with two outlet streams with lengths of 0.11 and 0.39 km. At times of extreme high tides (i.e. during the spring tide in September), this distance is minimized further, and there are reports that salt water may even enter the lake (Loewen 2008). Qasigiyat is an oligotrophic lake, clear and deep blue in colour.



Figure 2. Map of the Qasigiyat open lake system at the head of Ptarmigan Fiord.

Iqalugaarjuit (66°34'N, 66°43'W) connects to Shark Fiord near the head of Cumberland Sound (Fig. 1). The physiography of this lake is described in detail by Loewen (2008). In brief, the lake consists of upper and lower basins, covering areas of 0.15 and 1.11 km<sup>2</sup>, respectively (Fig. 3). Sampling is generally restricted to the lower basin for logistical reasons (Loewen 2008). Similar to Qasigiyat, the lower basin of Iqalugaarjuit reaches a maximum depth of approximately 20 m, but in contrast to Qasigiyat's steep edges, Iqalugaarjuit has gradually sloping sides. The single outlet stream is 0.30 km long, characterized by shallow braided channels running through boulders, with several deeper (~ 0.5 m) pools. Local knowledge reports that the river flow has decreased substantially in recent years, and in September 2009, after a summer of low precipitation, there was almost no water flowing from the river (J. Kakkik, pers. comm.). Unlike Qasigiyat, the maximum tide height does not broach the boundaries of the lake. Iqalugaarjuit is eutrophic, with turbid, brown-coloured water, aquatic plants, and an abundance of algae (Loewen 2008).



**Figure 3.** Map of the Iqalugaarjuit open lake system showing the two lake basins. Arctic char samples included in this study were collected from the lower lake basin, as indicated.

Both anadromous and resident Arctic char have been documented from these lakes (Loewen et al. 2009, DFO 2005). However, there are no known studies detailing their complete biological communities. Previous sampling and observations indicate that only one other fish species is present in these lakes, the threespine stickleback (*Gasterosteus aculeatus*) (Loewen 2008, DFO 2005).

# 2.2.2 Field Collections

Fishing took place in September 2011, following a similar protocol to that used to collect the archived data (i.e. samples collected in 2004) to maintain consistency between years, and followed an approved Animal Use Protocol (FWI-ACC-2011-046). Details of the 2004 sampling protocol can be found in Loewen (2008). In 2011, 38.1 mm and 63.5 mm stretched-mesh gillnets were set on the lake bottom, perpendicular from shore, and at average depths of 6.2 m (Qasigiyat) and 3.2 m (Iqalugaarjuit). Net locations were chosen

on the basis of previous sampling that yielded high proportions of resident Arctic char (S. Wiley, pers. comm.). The probability of capturing resident Arctic char was also maximized by setting and hauling nets at regular (~ 6 hour) intervals in the evenings and at night (Loewen 2008).

Upon capture, all Arctic char were sampled for biological data, including fork length (mm), weight (g), sex, and maturity status. Sagittal otoliths were removed, cleaned, and stored in a dry envelope. A plug of white muscle (approximately 2 x 5 cm) was cut from the dorsal surface of each fish, from a landmarked location immediately posterior to the head, anterior to the dorsal fin. All stomachs containing prey were removed from the oesophagus to the pyloric sphincter. Tissue samples and stomachs were placed in airtight bags, labelled, and stored in coolers immediately. Samples were frozen at -20°C as soon as possible, typically 3-5 days.

**Table 1.** Summary of Arctic char muscle samples analyzed from two open lake systems in the Cumberland Sound region in September 2004 and 2011, including sample size (*n*), mean fork length (L<sub>F</sub>), and mean ( $\pm$  SD) stable isotope ratios of carbon ( $\delta^{13}$ C), nitrogen ( $\delta^{15}$ N) and sulphur ( $\delta^{34}$ S) for adult anadromous (A) and resident (R), and immature (I) fish.  $\delta^{13}$ C and  $\delta^{34}$ S were derived from bulk (i.e. lipid containing) samples and  $\delta^{13}$ N was derived from lipid extracted samples.

Site	Year	Ecotype	n	L <sub>F</sub> (mm) δ <sup>13</sup> C		C (%	o)	δ <sup>18</sup>	⁵N ('	‰)	δ <sup>34</sup> S (‰)		
Qasigiyat	2004	А	11	550 ± 71	-20.1	±	0.4	13.7	±	0.6	18.4	±	0.3
	2004	R	9	167 ± 24	-22.6	±	1.6	9.8	±	1.2	9.5	±	3.3
	2011	А	21	604 ± 103	-19.5	±	0.4	14.1	±	0.5			
	2011	R	15	169 ± 30	-21.8	±	1.4	12.9	±	1.3			
	2011	I	20	182 ± 35	-20.7	±	1	13.6	±	1.5			
laalu-	2004	А	18	544 ± 45	-19.5	±	0.3	13.2	±	0.4	17.4	±	0.2
gaarjuit	2004	R	15	198 ± 23	-25.5	±	1.2	9.9	±	0.8	4.7	±	1.5
	2011	А	17	574 ± 69	-19.1	±	1.5	14.4	±	1.00			
	2011	R	11	176 ± 30	-24.8	±	1.6	10.0	±	1.5			
	2011		20	205 ± 33	-20.0	±	1.8	13.3	±	1.3			

Fish were tentatively placed in one of three ecotype categories: resident (R), anadromous (A), undeveloped. These identifications were based on fork length (L<sub>F</sub>, given in mm) and maturity status (mature, immature, resting, ripe, or spent; Gillman and Sparling 1985). Following a method adapted from Loewen (2008), resident fish were classified on the basis of small size (L<sub>F</sub> < 400 mm) and a mature, ripe, or spent maturity status. Fish were identified as anadromous if large (L<sub>F</sub>  $\geq$  400 mm) and mature, ripe, spent, or resting. Noting possible difficulties in distinguishing between immature and residents with a resting maturity status in the field, small (L<sub>F</sub> < 400 mm) fish that appeared immature were initially identified as having an undeveloped maturity status. Sampling in September allowed easier maturity status identification, as many mature fish were in spawning or pre-spawning condition. Fish whose ecotype affinity was ambiguous were also noted to determine if ecotype could be assigned using stable isotopes and fatty acids.

# 2.2.3 Archived Samples

In addition to samples collected in 2011, I analysed data and archived muscle tissue samples of Arctic char collected from Qasigiyat and Iqalugaarjuit in September 2004. These samples were collected as part of the Arctic char stock assessment program conducted by Fisheries and Oceans Canada.

Ecotypes were previously assigned to the archived samples on the basis of otolith strontium profiles, morphological characteristics, and life history (Loewen 2008). The trophic niche of Arctic char is known to shift with ontogenetic stage (Forseth et al. 1994, Eloranta et al. 2010, see Section 1.4); therefore, to control for ontogenetic differences among individuals, only clearly distinguishable adult fish (i.e. those with maturity status of mature, ripe, resting, and spent) were included in the analysis. The stable isotope ratios of these fish were compared to those from the 2011 samples to assess the validity of the qualitative ecotype designations.

# 2.2.4 Laboratory Analysis

# 2.2.4.1 Stable Isotope Analysis (SIA)

Approximately 3 g of muscle was subsampled from each frozen tissue sample and freeze-dried for a minimum of 48 hours (McMeans et al. 2010). The tissue was subsequently ground to a fine powder using a mortar and pestle and stored in a cryogenic vial. The high fat content of some samples prevented full homogenization; therefore, these samples were re-homogenized after lipid extraction (B. McMeans, pers. comm.). Lipids are a potentially confounding factor that must be accounted for when interpreting SIA results. Because lipids have relatively low  $\delta^{13}$ C in comparison to other tissue constituents, such as carbohydrates, the lipid quantity and variability among samples has

the potential to bias the results of carbon isotopic analyses (Post et al. 2007). The C:N ratios of bulk samples have been used as predictors of lipid levels, thus, high C:N ratios (< 4.0) indicate high levels of lipid content, necessitating accounting for lipids. In the absence of detailed species-specific validation, lipid extraction is preferred over *post hoc* mathematical normalization. However, it has been suggested that lipid extraction can lead to nitrogen isotope fractionation of 0.25 to 1.6‰ (Post et al. 2007, Mintenbeck et al. 2008). To address these issues, analysis of  $\delta^{15}$ N was conducted on bulk samples (i.e. non lipid-extracted), and analysis of  $\delta^{13}$ C was conducted on lipid extracted samples. The  $\delta^{34}$ S analysis was done on bulk samples, due to preliminary data suggesting that the lipid removal process may also have an impact on  $\delta^{34}$ S. The effect of lipid extraction on the isotopic profiles of Arctic char muscle is addressed in Chapter 4.

Homogenized samples were divided to allow bulk analysis and lipid extraction. Lipid extraction was done using a modified Bligh and Dyer (1959) method, as follows. Approximately 4 ml of 2:1 chloroform methanol (CHCl<sub>3</sub>:MeOH) solution was added to 0.5 ml of ground tissue. The sample was homogenized with a vortex mixer for 15 seconds and placed in a 30°C water bath. After 24 hours, the sample was centrifuged for 6 minutes, the supernatant was decanted and discarded, and another 4 ml of 2:1 CHCl<sub>3</sub>:MeOH was added. Again the sample was vortexed, centrifuged, and the supernatant discarded. The remaining solids were left to dry for 48 hours in a fume hood.

Samples were measured into pre-weighed tin capsules: 0.20-0.40 mg for  $\delta^{13}$ C and  $\delta^{15}$ N analysis, and 2.0-2.4 mg for  $\delta^{34}$ S analysis. Analyses of carbon and nitrogen isotopes were conducted simultaneously with a Delta V Advantage continuous-flow isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010

Elemental Combustion System (Costech Instruments, Valencia, CA, USA) at the Great Lakes Institute of Environmental Research (University of Windsor, Ontario). Every twelfth sample was run in triplicate, and four internal laboratory standards and one National Institute of Standards and Technology (NIST) reference standard were run after every 15 samples for quantification. The analytical precision (standard deviation) for NIST standard 8414 (bovine muscle, n = 103) and an internal lab standard (tilapia muscle, n = 103) for  $\delta^{13}$ C was 0.10 and 0.13‰, respectively, and for  $\delta^{15}$ N was 0.12 and 0.14‰, respectively.

Sulphur analysis was conducted with an Isochrom Continuous Flow Stable Isotope Ratio Mass Spectrometer (GVInstruments/Micromass, UK) coupled to a Costech Elemental Analyzer (CNSO 4010, UK). Two NIST organic sulphur materials, Bovine Liver and Mussel, were run for corrections. Every tenth sample was run in duplicate, with a standard error of  $\pm$  0.08‰. The NIST standards (sucrose and ammonia sulphate, *n* = 3) were within 0.01 and 0.07‰ of certified values for  $\delta^{15}$ N and  $\delta^{13}$ C, respectively.

In accordance with standard practices, stable isotope ratios are given as the difference in the isotopic ratio ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{34}$ S) between the sample and reference standards (Peterson and Fry 1987). The ratios are expressed as parts per thousand (‰) following the formula:

Eq. 1. 
$$\delta^{13}C, \ \delta^{15}N, or \ \delta^{34}S = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000$$

where R = the ratio of carbon (<sup>13</sup>C: <sup>12</sup>C), nitrogen (<sup>15</sup>N: <sup>14</sup>N), or sulphur (<sup>34</sup>S: <sup>32</sup>S) isotopes of the sample. By convention, standards are set at 0‰. The change ( $\Delta$ ) in each variable following lipid extraction was calculated as:

# Eq. 2. $\Delta \mathbf{X} = \mathbf{X}_{LE} - \mathbf{X}_{E}$

where  $X = \delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^{34}S$ , or C:N; LE = lipid extracted; and B = bulk.

Baseline levels of  $\delta^{13}$ C and  $\delta^{15}$ N can be variable between and within ecosystems, introducing potential bias when interpreting trophic positioning and comparing among systems (Vander Zanden et al. 1997, Post et al. 2002). Therefore, comparisons between systems and between years were considered on a relative scale only (i.e. absolute trophic position could not be calculated).

## 2.2.4.2 Fatty Acid Analysis

Lipids were extracted following a modified Folch et al. (1957) procedure, and all extractions were conducted within two months of sample collection to minimize the risk of fatty acid oxidation (Budge et al. 2006). Approximately 2 g of muscle tissue was subsampled from the centre of the sample and trimmed to remove damaged and possibly oxidized tissue. The tissue was freeze-dried for  $\geq$  48 hours (Elsdon 2010, Heissenberger et al. 2010). Following freeze-drying the tissue was homogenized by mortar and pestle, and a 0.5 g subsample (to nearest 0.01 g) was placed in a clean microcentrifuge tube. To the subsample, 3 ml MeOH with 0.01% BHT and 6 ml CHCl<sub>3</sub> were added, and the sample was stored at -40°C for 12 to 24 hours.

After 24 hours, 2 ml of 2:1 CHCl<sub>3</sub>/MeOH was added to extract the lipids, 7 ml 0.88% NaCl was added to remove unwanted components from the sample, and NaSO<sub>4</sub> was added to remove the water. The remaining solvent was evaporated with gaseous nitrogen in a water bath, leaving the lipids, which were weighed to the nearest 0.001 g.

Transesterification of the lipids began with the addition of 1.5 ml dichloromethane (DCM) with 0.01% BHT, followed by the addition of 3.0 ml Hilditch reagent (1.5 ml

 $H_2SO_4$  and 100 ml dry methanol). After flushing with gaseous nitrogen and mixing, the samples were heated for 1 hour at 100°C. The samples were allowed to cool to room temperature and then 3 ml hexane and 1 ml distilled water were added to produce fatty acid methyl esters (FAME). Any remaining water was precipitated with NaSO<sub>4</sub>. Once again, the solvent was evaporated under nitrogen in a water bath, and the total FAME weight was measured (to nearest 0.001 g). Hexane was added to produce a concentration of 100 mg FAME per ml hexane, and 1.0 ml of this solution was transferred to a gas chromatography vial, flushed with nitrogen, and sealed.

Fatty acids were identified using an Agilent 7890A GC (Agilent Technologies) equipped with a flame ionization detector (FID) and a 30m DB-23 column (ID 0.25 mm, film thickness 0.25 μm). Samples were injected in volumes of 1 μL by an autosampler. The injector was run in pulsed splitless mode at 260°C with an injection pressure pulse of 50 psi for 1.25 min. Hydrogen was used as carrier gas at a flow rate of 1.4 mL min<sup>-1</sup>. The GC temperature program began at 60°C with a 1 minute hold, increased to 165°C at 15 °C min<sup>-1</sup> with a 3 min hold, ramped up to 174°C at a rate of 3°C min<sup>-1</sup>, and then increased to 200°C at a rate of 5 °C min<sup>-1</sup> and was held for an additional 9 min. The FID temperature was 350°C and the fuel gas mixture was 30 mL min<sup>-1</sup> hydrogen and 400 mL min<sup>-1</sup> zero air. A 37 component FAME standard (Supelco) was used to make a 4-point calibration curve (50, 100, 200 and 500 ng). A 200 ng standard was run after every ten samples, and every tenth sample injection was duplicated.

Fatty acids were identified from the chromatograms using GC ChemStation Rev. B.03.02 (Agilent Technologies) by comparing to a reference standard mixture (Supelco 37 component FAME mix and Nu-Check Prep GLC-463). Baselines were manually set

and peaks were manually integrated (Budge et al. 2006). Individual fatty acid data are presented as a percentage of the total percent fatty acid in the sample based on peak areas. Fatty acids representing < 1% of the total fatty acids were excluded from the analysis (Stowasser et al. 2009, Loseto et al. 2009). Fatty acids identities are given in the form of C22:6n-3, where C22 denotes the number of carbon atoms, followed by the number of double bonds (here, 6) and the location of the first double bond in the molecule (n-3).

# 2.2.5 Data Analysis

Exploratory and univariate statistical analysis was conducted using SigmaStat v. 3.5 (Systat Software Inc. 2006), and multivariate analysis was done with R 2.12.1 (R Core Development Team 2008). Significance was set at 0.05. Sexes were pooled for all analyses on the basis of previous work showing no differences in diet between male and female Arctic char (Isinzugo 2009). The parametric assumptions of normality and homogeneity of variances were tested with the Kolmogorov-Smirnov test and examination of residuals (Zar 1999). When these basic assumptions were not met, data were log transformed and reassessed. The non-parametric equivalent test was used when transformation failed to normalize the data (e.g. Wilcoxon signed rank, Kruskal-Wallis ANOVA on Ranks, Mann-Whitney U; Zar 1999).

# 2.2.5.1 Stable Isotope Analysis (SIA)

The size frequencies for each ecotype and the relationship between  $L_F$  and  $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta^{34}S$  were assessed, because diet of Arctic char can vary with fish size (Moore and Moore 1974, Dempson et al. 2002). For each study site, data for both years were combined for assessment of size effects on isotopic signature. One-way ANOVAs were

used to determine significant differences in fork length among groups (resident, anadromous, and immature) within each site. Linear regressions were applied to assess the relationship of fork length with stable isotope signature, with ecotype groups and sites treated separately.

Stable isotope analysis results were interpreted visually from dual isotope plots of  $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S (Kwak and Zedler 1997, McCarthy and Waldron 2000). ANOVAs were used to compare  $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S isotopic ratios among groups within each study system. *Post hoc* comparison between groups was done with Tukey's HSD test. Student's *t*-tests were used to compare stable isotope ratios of the resident and anadromous fish between sampling years. Data from stable isotope analysis were left untransformed, because exploratory data analysis showed that transformations did not effectively normalize the data.

# 2.2.5.2 Fatty Acid Analysis

Fatty acid profiles were analysed to determine possible differences in the muscle fatty acid composition of Arctic char ecotypes. One-way ANOVAs were applied to untransformed fatty acid data to assess differences in the proportions of specific individual fatty acids among anadromous, resident and immature fish. Tukey's HSD test was used to conduct pair-wise comparisons when significant differences were found.

Fatty acid percentages were transformed using the centred log ratio transformation to allow for classical multivariate analysis (Aitchison 1982, Loseto et al. 2009). Principal components analysis (PCA) was based on a covariance matrix of the data, and was used to evaluate differences in fatty acid composition among ecotype groups. The PCA loadings were assessed for the fatty acids and fatty acid groups with the largest

contribution to the overall variance.

## 2.3 Results

## 2.3.1 Field Collections

Arctic char belonging to the three predetermined categories were captured during the 2011 field season (Table 1). From Qasigiyat a total of 132 Arctic char were captured: 15 residents, 41 anadromous, and 70 undeveloped. Six fish were categorized as ambiguous. From Iqalugaarjuit, 78 fish were captured: 11 residents, 17 anadromous, and 47 undeveloped. Three fish were categorized as ambiguous. Residents from Iqalugaarjuit were readily identified by a brown colouration, truncated head shape, and larger eyes, although these descriptors were not quantified in the present study. The extent of the differences allowed classification of five immature fish as residents. In contrast, Qasigiyat residents were less easily distinguished, with less apparent colouration and morphological differences. Qasigiyat residents were silvery in colouration with pale spots, making them visually similar to immature anadromous fish. Reference photographs of anadromous and resident Arctic char captured from Iqalugaarjuit and Qasigiyat during these field collections are given in Appendix E. A large number of threespine sticklebacks (Gasterosteus aculeatus) and chironomid larvae were observed within the Iqalugaarjuit lake proper during sampling activities.

Most fish were not feeding at the time of sampling, as few stomachs contained prey. From the fish sampled, 93% (Qasigiyat) and 83% (Iqalugaarjuit) had empty stomachs. Stomach contents were not formally assessed, but instances of cannibalism were observed at both study sites. In Qasigiyat, a young-of-the-year (fork length = 50 mm) conspecific was found in the mouth of a  $L_F = 181$  mm mature resident Arctic char.

Conspecific eggs were found in the stomachs of two individuals – both males – one of which was a  $L_F = 438$  mm resting anadromous fish, the other a  $L_F = 204$  mm mature resident.

## 2.3.2 Stable Isotopes

Resident and anadromous Arctic char had significantly different stable isotope ratios. The previously identified anadromous and resident fish captured in 2004 formed distinct groups on the dual isotope plots (Fig. 4a-c), with the most striking ecotype differentiation when  $\delta^{15}$ N and  $\delta^{34}$ S were plotted together (Fig. 4c). All three isotopes were significantly lower in resident fish than anadromous fish (P < 0.05 in all cases). Statistically significant differences are summarized in Table 2. The fish captured in 2011 followed the same pattern: anadromous fish had significantly higher  $\delta^{13}$ C and  $\delta^{15}$ N compared to residents (P < 0.05 in both cases; Fig. 5). The relationship between immature fish stable isotope ratios with anadromous and resident fish was different in the two systems. In Qasigiyat, the  $\delta^{13}$ C of immature fish was significantly lower compared to anadromous fish, but was not significantly different from residents (Fig. 5a). Immature fish did not have significantly different  $\delta^{15}$ N than either anadromous or resident Arctic char in Qasigiyat. In Iqalugaarjuit, immature fish formed a distinct group, with  $\delta^{13}C$  and  $\delta^{15}N$  of immature fish both significantly different from anadromous and resident fish (Fig. 5b). For immature fish from Iqalugaarjuit, the stable isotope ratios were higher relative to residents and lower than anadromous fish (Table 1).

For pooled data (i.e. both systems and years), the mean difference in isotopic ratios between anadromous and resident Arctic char was 3.2‰ for  $\delta^{15}$ N, 4.1‰ for  $\delta^{13}$ C, and 10.8‰ for  $\delta^{34}$ S. The range and standard deviation of stable isotope ratios of the resident

ecotype was consistently larger than for the anadromous fish (Table 1).

The degree of similarity between ecotypes – with respect to their stable isotope ratio – was different within the systems. As seen in Fig. 5, the ecotypes from Qasigiyat were relatively similar to each other, as opposed to the large difference in stable isotope ratios between ecotypes from Iqalugaarjuit. In the 2004 dataset, the range of  $\delta^{13}$ C in Qasigiyat resident (-19.8 to -24.4‰) and anadromous (-20.3 to -23.1‰) overlapped, whereas in Iqalugaarjuit there was a discrete difference in  $\delta^{13}$ C between ecotypes ( $\delta^{13}$ C<sub>resident</sub> = -22.0 to -27.1‰;  $\delta^{13}$ C<sub>anadromous</sub> = -18.9 to -20.0‰). There was also an overlap in  $\delta^{15}$ N from the Qasigiyat 2011 dataset, which is also evident in the shift of  $\delta^{15}$ N of residents toward that of anadromous fish (Fig. 5a). In Iqalugaarjuit, two anadromous fish had stable isotope signatures approaching those of residents (Fig 5b).

The stable isotope ratios for resident fish did not differ significantly between 2004 and 2011, except for the residents of Qasigiyat, for which  $\delta^{15}$ N was significantly higher in 2011 (t = -5.736, df =22, P < 0.001). Anadromous fish from both study sites had higher  $\delta^{13}$ C in 2011 compared to 2004 (Qasigiyat: t = -4.532, df = 30, P < 0.001; Iqalugaarjuit: Mann-Whitney U = 37.0,  $n_1 = 17$ ,  $n_2 = 18$ , P < 0.001). Only anadromous fish from Iqalugaarjuit had significantly higher  $\delta^{15}$ N (t = -5.032, df = 33, P < 0.001) in 2011 compared to 2004.

The fish with ambiguous morphology from Qasigiyat were immature, and stable isotope analysis placed these fish within the range of other immature fish, although one exhibited  $\delta^{13}$ C more similar to residents and one grouped closely with anadromous fish (Fig. 5a). In Iqalugaarjuit, one ambiguous fish was within the resident grouping, whereas the other two had somewhat higher  $\delta^{15}$ N and had  $\delta^{13}$ C closer to residents (Fig. 5b).

**Table 2.** Significance (*P*-values) of statistical testing (*t*-tests and Mann-Whitney rank sum) for differences between ecotypes and sites in the carbon and nitrogen stable isotope ratios ( $\delta^{13}$ C and  $\delta^{15}$ N) of resident (R) and anadromous (A) Arctic char from the two study sites (Qasigiyat and Iqalugaarjuit). Data are for 2004 samples only. Results for  $\delta^{13}$ C are given in the upper right and  $\delta^{15}$ N are given in the upper left.

		Qas	sigiyat	Iqalu	ıgaarjuit
		Resident	Anadromous	Resident	Anadromous
Qasigiyat	R		0.003	0.001	
	А	0.001			0.001
Iqalugaarjuit	R	0.34			0.001
	А		0.001	0.004	



**Figure 4.** Dual isotope plots of (a)  $\delta^{13}$ C and  $\delta^{15}$ N, (b)  $\delta^{13}$ C and  $\delta^{34}$ S, and (c)  $\delta^{15}$ N and  $\delta^{34}$ S from muscle tissue of anadromous and resident Arctic char captured from two open lake systems (Qasigiyat and Iqalugaarjuit) in September 2004. The mean (± SEM) of each group are represented by large symbols.



**Figure 5.** Individual  $\delta^{13}$ C and  $\delta^{15}$ N of anadromous, resident, and undeveloped Arctic char captured in 2011 from two open lake systems in Cumberland Sound region of Nunavut: (a) Iqalugaarjuit and (b) Qasigiyat. The  $\delta^{15}$ N and  $\delta^{13}$ C were analyzed from bulk and lipid extracted samples, respectively. Means (± SEM) for 2004 and 2011 data are represented by large symbols.

# 2.3.3 Fork Length and Stable Isotopic Signature

The fork lengths (L<sub>F</sub>) of mature resident and anadromous Arctic char captured in both study sites were distinctly bimodal with no overlap in the sample populations in 2011 (Fig. 6). Residents fell within the lower mode, ranging in  $L_F$  from 107 to 226 mm in Qasigiyat and 123 to 254 mm in Iqalugaarjuit. The upper mode comprised of mature anadromous Arctic char, with L<sub>F</sub> ranging from 402 to 839 mm (Qasigiyat) and 455 to 704 mm (Iqalugaarjuit). The fork lengths of undeveloped fish were mostly included in the lower mode (Qasigiyat: 107-342 mm; Iqalugaarjuit: 134 - 290 mm), although there was more of a continuum observed in Qasigiyat (Fig. 6). Undeveloped fish had significantly smaller fork lengths than mature anadromous fish (P < 0.001), but no difference was observed in fork length between resident and immature fish in either system (Fig. 6). The fork lengths of all ambiguous fish captured in Qasigiyat were within the range of both immature and resident fish, whereas in Iqalugaarjuit, two of the ambiguous fish had fork lengths more similar to anadromous fish. The fork lengths of individuals of each ecotype randomly selected for stable isotope analysis are shown in Fig. 7. The two study years were pooled, because there were no significant differences in fork length between years. All undeveloped fish included in the subsequent analyses were smaller than the size-atmaturity range reported for these systems (Qasigiyat  $L_F = 290$  to 745 mm, Iqalugaarjuit  $L_F = 370$  to 680 mm; Loewen et al. 2010); therefore, these undeveloped fish were all considered to be immature.

There was a site-specific difference in  $L_F$  for residents, but not for anadromous fish. Resident fish captured from Qasigiyat were significantly smaller than those captured from Iqalugaarjuit (t = -2.654, df = 48, P = 0.011), whereas the size of anadromous fish

did not differ significantly between sites (t = 1.403, df = 65, P = 0.166).

The relationship between stable isotope ratios and fork length (L<sub>F</sub>) was variable between ecotypes and between systems. The  $\delta^{13}$ C was positively correlated with fork length only for anadromous Arctic char from Iqalugaarjuit (r = 0.493, n = 35, P = 0.003), and resident fish from Qasigiyat (r = 0.625, n = 25, P = 0.001) (Fig. 8). The trend for  $\delta^{15}$ N was more consistent: anadromous fish from both systems had higher  $\delta^{15}$ N with longer fork lengths (Qasigiyat: r = 0.703, n = 32, P < 0.001; Iqalugaarjuit, r = 0.493, n = 35, P < 0.001). Resident  $\delta^{15}$ N did not correlate with L<sub>F</sub>, and neither  $\delta^{13}$ C nor  $\delta^{15}$ N were associated with L<sub>F</sub> in immature fish. The sulphur stable isotope ratio ( $\delta^{34}$ S) was not correlated with fork length for either ecotype or location. I did not account for fish body size in subsequent statistical tests due to the lack of a consistent relationship between L<sub>F</sub> and  $\delta^{13}$ C or  $\delta^{15}$ N, and no relationship with  $\delta^{34}$ S. In addition, this research is focused on the between-group relationships, and there is little overlap between anadromous and resident/immature groups.



**Figure 6.** Fork length ( $L_F$ , mm) frequencies for all Arctic char captured from (a) Qasigiyat and (b) Iqalugaarjuit open lake systems in 2011.



**Figure 7.** Fork length ( $L_F$ , mm) frequency histograms of Arctic char included in stable isotope analysis from (a) Qasigiyat and (b) Iqalugaarjuit open lake systems. Study years (2004 and 2011) were pooled.



**Figure 8.** Relationship between fork length (mm) and (a) carbon stable isotope ratio  $(\delta^{13}C)$ , (b) nitrogen stable isotope ratio  $(\delta^{15}N)$ , and (c) sulphur stable isotope ratio  $(\delta^{34}S)$  for Arctic char from Qasigiyat and Iqalugaarjuit in the Cumberland Sound region. Trendlines are shown only where statistically significant correlations exist:  $\delta^{13}C$  of Qasigiyat residents (solid line; r = 0.625, P = 0.001) and Iqalugaarjuit anadromous (dashed line; r = 0.493, P = 0.003), and  $\delta^{15}N$  of anadromous fish from Qasigiyat (solid line; r = 0.703, P < 0.001) and Iqalugaarjuit (dashed line; r = 0.708, P < 0.001).

#### 2.3.4 Fatty Acids

Of the 70 fatty acids that were regularly identified, 22 were chosen for further analysis because of biological or quantitative significance (i.e. > 1% of the total fatty acids; Gladyshev et al. 2010). The fatty acid compositions of the three groups (anadromous, resident, and immature) for each study system are given in Table 3. The fatty acids with the highest proportions were C16:0, C16:1n7, C18:1n9, C20:5n3 (EPA), and C22:6n3 (DHA), representing 61-71% of the total fatty acid composition in all groups; however, the proportions of each were variable among group and study site. Polyunsaturated fatty acids (PUFA) made the greatest contribution (41-49%) to fatty acid type in resident and immature fish, whereas monounsaturated fatty acids (MUFA) were the fatty acid type with the highest proportion in anadromous fish (34-37%).

Results of principal components analysis showed distinct separation of anadromous and resident Arctic char (Fig. 9). This separation occurred primarily along the first PCA axis (PCA1) owing to a suite of fatty acids that were similar between the two systems (Fig. 10 and Fig. 11). The PCA1 variation was mostly influenced by PUFAs (Qasigiyat: ARA and DHA, Iqalugaarjuit: ARA and LIN) and SFAs (C16:0 and C18:0), that were negatively correlated with MUFAs (C20:1 and C22:1; Table 4). The most influential fatty acids contributing to the variation along the second axis (PCA2) differed between the two systems. EPA contributed most to explaining the variance for Qasigiyat, whereas ALA and DHA (which were negatively correlated) were influential for Iqalugaarjuit (Table 4). For both systems, C16:1n7, C18:1n9, and MUFAs in general (particularly C18:1) were important for separation along PCA2 (Fig. 9). The proportion of variance accounted for by the first two axes was 78.6% for Qasigiyat (PCA1: 65.0%, PCA2: 13.6%; Fig. 10a),

and 72.2% for Iqalugaarjuit (PCA1: 58.0%, PCA2: 14.1%; Fig. 11a). Immature fish from Qasigiyat did not form a distinct group; rather, immature fish grouped with either anadromous or resident Arctic char (Fig. 10a). Iqalugaarjuit immature fish formed a loose grouping close to the anadromous group, separated from the resident ecotype along PCA1, and separated from the anadromous group along PCA2 (Fig. 11a). Classification of ambiguous fish using fatty acid profiles was only possible in some instances. From Iqalugaarjuit, one ambiguous fish had a fairly distinct resident-type fatty acid signature (Fig. 11a). In contrast, the other two ambiguous fish from Iqalugaarjuit were large (< 400mm) resting males, and exhibited fatty acid profiles different from both resting and anadromous fish, although PCA grouped them furthest from the anadromous fish. Two of the ambiguous fish from Qasigiyat grouped with resident or immature fish, and the third grouped with anadromous or immature fish. Fork length did have a consistent influence on fatty acid profiles, besides the difference between the smallest and the largest fish, which corresponded to the resident and anadromous ecotypes, respectively (Fig. 10b and 11b).

The differences observed between groups from PCA were supported by ANOVA results (Table 3). MUFA were significantly higher in anadromous fish compared to both resident and immature fish, whereas polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) were significantly lower in resident and immature fish. DHA was significantly lower in anadromous fish than in both resident and immature fish. The proportion of EPA was not consistent for group type between systems: anadromous fish from Qasigiyat had significantly higher EPA than resident fish, but no significant difference compared to immature fish. Yet EPA in anadromous fish from Iqalugaarjuit

was significantly different than in immature fish, but not when compared to residents. EPA was the only fatty acid that was significantly different between resident and immature fish from Qasigiyat. Overall, in fish from Qasigiyat there were significant differences between anadromous and resident ecotypes for 18 of the 22 fatty acids, whereas 15 of the 22 fatty acids were significantly different between the anadromous and immature groups (Table 3). The same trend emerged between resident and anadromous fish in Iqalugaarjuit: 19 of the individual fatty acids were significantly different between these categories, and eight of the fatty acids between anadromous and immature fish (Table 3). However, there was a greater difference between resident and immature fish in Iqalugaarjuit compared to Qasigiyat, as there were significant differences in 12 and two fatty acids, respectively.

	Qasigi	yat				•					Iqaluga	aarj	uit							
Fatty Acid	A ( <i>n</i> =	8)		R ( <i>n</i> =	: 10	)	l ( <i>n</i> = '	10)			A ( <i>n</i> =	10)		R ( <i>n</i> =	10)		l ( <i>n</i> = 9	)		
C14:0	3.2	±	0.2	1.1	±	0.2	1.7	±	0.3	*	3.2	±	0.3	1.4	±	0.2	2.6	±	0.3	Ť
C16:0	13.1	±	0.4	17.2	±	0.3	16.8	±	0.6	*	14.4	±	0.8	17.3	±	0.3	16.5	±	0.4	*
C16:1n7	10.7	±	0.3	5.7	±	1.0	6.1	±	0.8	*	13.5	±	1.3	7.1	±	0.5	7.1	±	0.8	*
C18:0	1.9	±	0.1	4.2	±	0.3	3.5	±	0.2	*	2.7	±	0.2	4.5	±	0.1	3.2	±	0.2	Ť
C18:1n11	1.0	±	0.0	0.4	±	0.1	0.5	±	0.1	*	0.4	±	0.1	0.1	±	0.0	0.4	±	0.1	
C18:1n9	12.4	±	0.6	10.1	±	0.9	10.4	±	1.1		16.3	±	1.1	11.1	±	0.6	11.0	±	0.6	*
C18:1n7	3.1	±	0.1	3.7	±	0.2	3.3	±	0.2		4.1	±	0.1	4.7	±	0.2	3.6	±	0.1	*†
C18:1n5	0.7	±	0.0	0.3	±	0.0	0.6	±	0.1	†	0.4	±	0.0	0.3	±	0.0	0.5	±	0.0	Ť
C18:2n6 (LIN)	0.8	±	0.0	4.3	±	0.8	2.5	±	0.8		1.5	±	0.2	6.0	±	0.7	0.7	±	0.1	Ť
C18:3n3 (ALA)	0.3	±	0.0	1.3	±	0.2	0.9	±	0.2		0.4	±	0.1	2.5	±	0.3	0.3	±	0.0	Ť
C18:4n3	1.5	±	0.1	0.3	±	0.0	0.6	±	0.1	*	0.9	±	0.1	1.2	±	0.1	1.0	±	0.1	
C20:1n11	1.1	±	0.1	0.1	±	0.0	0.2	±	0.1	*	0.4	±	0.1	0.1	±	0.0	0.2	±	0.1	*
C20:1n9	7.2	±	0.5	1.1	±	0.2	2.3	±	0.6	*	4.4	±	0.9	0.8	±	0.2	3.0	±	0.7	
C20:1n7	0.8	±	0.1	0.2	±	0.0	0.3	±	0.1	*	0.6	±	0.1	0.2	±	0.0	0.4	±	0.1	*†
C20:3n6	0.2	±	0.0	0.5	±	0.1	0.6	±	0.3		0.2	±	0.0	0.5	±	0.1	0.1	±	0.0	†
C20:4n6 (ARA)	0.5	±	0.0	2.8	±	0.3	2.2	±	0.6	*	1.1	±	0.6	3.5	±	0.4	0.7	±	0.1	Ť
C20:4n3	1.1	±	0.0	0.6	±	0.1	0.7	±	0.1	*	0.9	±	0.1	1.1	±	0.1	0.8	±	0.1	
C20:5n3 (EPA)	12.5	±	0.6	7.4	±	0.3	11.5	±	1.0	t	10.0	±	0.5	9.0	±	0.6	13.6	±	0.7	*†
C22:1n11	6.0	±	0.5	0.3	±	0.1	1.7	±	0.7	*	2.4	±	0.6	0.3	±	0.2	2.1	±	0.6	Ť
C22:1n9	1.4	±	0.1	0.2	±	0.0	0.4	±	0.1	*	0.6	±	0.1	0.2	±	0.0	0.5	±	0.1	Ť
C22:5n3	3.0	±	0.2	3.2	±	0.2	2.9	±	0.2		3.5	±	0.2	2.4	±	0.3	2.7	±	0.3	
C22:6n3 (DHA)	12.1	±	0.7	29.2	±	2.7	24.5	±	2.5	*	12.2	±	1.6	18.2	±	1.6	22.4	±	1.9	*
SUM SFA	18.9	±	0.4	23.2	±	0.5	22.8	±	0.6	*	21.0	±	0.8	24.3	±	0.3	23.1	±	0.4	*
SUM MUFA	34.7	±	1.1	18.9	±	1.9	21.3	±	2.1	*	37.0	±	2.6	21.1	±	1.1	23.7	±	1.7	*
SUM PUFA	29.9	±	1.4	49.6	±	2.2	45.8	±	2.8	*	29.5	±	2.4	43.4	±	1.3	41.1	±	2.4	*

**Table 3.** Mean ( $\pm$  SD) fatty acid composition (% total fatty acids) of anadromous (A), resident (R), and immature (I) Arctic char from two lake systems in the Cumberland Sound region. Statistically significant differences (P < 0.05) for individual fatty acids between groups (One-way ANOVA) are given by A vs R (bold typeface), A vs I (\*), and R vs I (†) for fish within each site.

**Table 4.** Factor loadings for fatty acid contributions to the first two principal component axes contributing to 78.6, 72.2, and 69.5% of the variance for Qasigiyat, Iqalugaarjuit, and both sites pooled, respectively. Data are for Arctic char muscle samples analyzed from two open lake systems (Qasigiyat and Iqalugaarjuit) in the Cumberland Sound region.

	Qasig	iyat	Iqaluga	arjuit	Pooled			
	PCA 1	PCA 2	PCA 1	PCA 2	PCA 1	PCA 2		
C14:0	-0.19	0.21	0.19	-0.26	-0.17	-0.34		
C16:0	0.23	-0.08	-0.23	0.25	0.26	0.15		
C16:1n7	-0.15	0.38	0.09	-0.43	-0.09	-0.39		
C18:0	0.23	-0.08	-0.25	0.15	0.26	0.12		
C18:1n5	-0.16	-0.20	0.17	0.23	-0.17	0.22		
C18:1n7	0.20	0.16	-0.24	-0.09	0.24	-0.13		
C18:1n9	0.08	0.28	0.06	-0.30	0.08	-0.20		
C18:1n11	-0.18	-0.19	0.22	0.18	-0.20	0.30		
C18:2n6 (LA)	0.20	0.26	-0.25	-0.15	0.25	-0.13		
C18:3n3 (ALA)	0.20	0.24	-0.27	-0.10	0.26	-0.13		
C18:4n3	-0.22	0.03	-0.11	-0.39	-0.08	-0.43		
C20:1n7	-0.18	-0.08	0.25	0.02	-0.22	0.01		
C20:1n9	-0.23	-0.05	0.27	0.02	-0.28	0.05		
C20:1n11	-0.23	-0.06	0.27	-0.06	-0.27	0.02		
C20:3n6	0.18	0.18	-0.25	-0.10	0.24	-0.02		
C20:4n3	-0.10	-0.10	-0.21	-0.21	0.12	-0.32		
C20:4n6 (ARA)	0.24	0.01	-0.26	0.13	0.27	0.08		
C20:5n3 (EPA)	0.02	-0.41	-0.05	0.20	0.06	0.14		
C22:1n9	-0.23	-0.06	0.27	0.05	-0.28	0.05		
C22:1n11	-0.23	-0.09	0.25	0.13	-0.27	0.07		
C22:5n3	0.17	-0.28	-0.04	0.15	0.06	0.21		
C22:6n3 (DHA)	0.22	-0.17	-0.17	0.39	0.22	0.31		


**Figure 9.** Principal components analysis revealing the fatty acids contributing to differences between anadromous (A), resident (R), immature (I), and ambiguous (U) Arctic char from two open lake systems (Qasigiyat = PG015; Iqalugaarjuit = PG082) within the Cumberland Sound region.



**Figure 10.** Principal components results of fatty acid composition of Arctic char muscle tissue collected in September 2011 from Qasigiyat. Individuals are grouped by (a) ecotype and (b) fork length (mm).



**Figure 11.** Principal components results of fatty acid composition of Arctic char muscle tissue collected in September 2011 from Iqalugaarjuit. Individuals grouped by (a) ecotype and (b) fork length.

# **2.4 Discussion**

Anadromous and resident Arctic char occupy different trophic niches, as shown by results from stable isotope and fatty acid analyses that indicate anadromous feeding in the marine environment and resident feeding in fresh water, in accordance with previous investigations of these populations (Loewen et al. 2009) and with other Arctic char research (Swanson et al. 2010). There is some indication of resident use of the estuarine environment in Qasigiyat, as stable isotope and fatty acid signatures suggest use of marine resources, and this strategy might be due to an opportunity to exploit a new trophic niche or minimization of intraspecific competition in the lake. Immature fish appear to have a separate trophic niche from both residents and anadromous ecotypes, although similarities exist.

This study has validated the use of stable isotope profiles as a classification tool for determining anadromous and resident ecotypes of Arctic char. For this study, it was important to first ascertain whether resident and anadromous ecotypes could be confirmed by stable isotope ratios using previously identified fish prior to assigning ecotype status of captured fish with isotopic ratios alone. Ecotype identities that were previously assigned on the basis of a combination of otolith strontium levels and maturity status (Loewen 2008) were confirmed here by stable isotope profiles. One isotope alone would not be sufficient to distinguish between the ecotypes, but carbon and nitrogen, sulphur and nitrogen, or ideally all three isotopes, can provide a clear representation of migratory ecotypes. One of the first applications of stable isotopes to assign migratory type was for broad whitefish (*Coregonus nasus*) by Hesslein et al. (1993), and has since been successfully applied to other salmonids, including brook trout (*Salvelinus fontinalis*)

(Doucett et al. 1999), brown trout (McCarthy and Waldron 2000), and rainbow trout (Ciancio et al. 2008). Recently, Swanson et al. (2010) documented anadromy in lake trout for the first time, from the West Kitikmeot region of the Canadian Arctic, in part by assessing the stable isotope ratios of the lake trout and comparing to them to known partially migratory Arctic char.

The stable isotope profiles of anadromous and resident fish identified in this study show that fish with higher  $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S ratios were those that employed an anadromous strategy, whereas those with lower stable isotope ratios tended to be nonmigratory. The stable isotope ratios of Arctic char found here closely resemble those characterized by Swanson et al. (2010) in the Kitikmeot region of Nunavut and Doucett et al. (1999) in Québec.

The baseline carbon isotope ratio range in the marine environment generally falls between -19 and -24‰, reflecting marine planktonic production (Peterson and Fry 1987). The stomach contents of anadromous Arctic char in the Cumberland Sound region identified in Chapter 3 of this thesis has confirmed the marine trophic niche of anadromous Arctic char, showing that marine fishes and amphipod invertebrates were the most important diet items. The  $\delta^{13}$ C for anadromous Arctic char found in this study is similar to values found in other marine pelagic fish in Cumberland Sound (Marcoux et al. 2012), providing evidence that these fish are feeding in the marine environment. Marcoux et al. (2012) reported capelin had a  $\delta^{13}$ C value of -19.52 ± 0.26‰, and Arctic cod (*Boreogadus saiga*) had a  $\delta^{13}$ C value of -19.86 ± 0.48‰. Cumberland Sound Arctic char stable isotope values have also been reported by Marcoux et al. (2012), and these values ( $\delta^{13}$ C: -18.64‰ and  $\delta^{15}$ N: 14.06‰) are very similar to my findings. However, a direct comparison is compromised because fish in the Marcoux et al. (2012) study were captured opportunistically in 2007 and 2009 and specific capture locations were not reported, and there are potential differences in stable isotope profiles at different times of year and among locations.

The  $\delta^{34}$ S identified for anadromous fish approaches the  $\delta^{34}$ S of marine phytoplankton, generally considered to be +20-21‰, reflecting sulfate availability in sea water (Peterson and Howarth 1987, Godbout et al. 2010). The  $\delta^{34}$ S of fresh water organisms is generally considered to be more variable, but in general lower than marine (Godbout et al. 2010), which is aligned with the patterns observed here for resident Arctic char.

Variability in the type and contribution of carbon sources (e.g. mineral weathering, terrestrial vegetation) can result in high variability in carbon isotope ratios among freshwater systems, but in general, freshwater systems have low  $\delta^{13}$ C compared to marine systems (Peterson and Fry 1987). The lower  $\delta^{13}$ C of resident fish compared to anadromous fish suggests that residents inhabit a trophic niche within the fresh water environment. No published  $\delta^{13}$ C values exist for fresh water in this region; however, reports of  $\delta^{13}$ C from resident Arctic char in other regions ranges from approximately - 29.2 to -19.6‰ (Doucett et al. 1999, Swanson et al. 2010). The wide range in these reported carbon values may be accounted for by differences in baseline carbon signatures among study locations.

The larger ranges of both  $\delta^{13}$ C and  $\delta^{15}$ N found in resident fish compared to anadromous fish mirror the results of Ciancio et al. (2008) from salmonids (rainbow trout and brown trout) in Patagonia. The higher variation in resident  $\delta^{13}$ C may be caused by a

wider range in baseline  $\delta^{13}$ C present in fresh water. There is well-documented  $^{13}$ Cdepletion in freshwater phytoplankton compared to terrestrial vegetation (Rau 1978, Fry and Sherr 1984) that would lead to a wider range of source  $\delta^{13}$ C in the freshwater food chain. Both freshwater zooplankton and terrestrial insects are available to Arctic char in the freshwater regions of these systems (Moore and Moore 1974), and both landlocked and anadromous individuals are documented to make use of these resources (Power et al. 2002, Svenning et al. 2007, see Section 3.3.1). A large range in  $\delta^{15}$ N is generally considered to be an indicator of wider niche width (Bearhop et al. 2004). In a simultaneous comparison of anadromous and lake-dwelling juvenile Arctic char, Rikardsen et al. (2000) found that the anadromous fish had a narrower diet width than the lake-dwellers that fed on a larger variety of diet items. Resident Arctic char from my study lakes are likely feeding on a wider range of prey items, receiving carbon from both terrestrial and freshwater primary carbon sources, and nitrogen from a wider range of trophic levels. In contrast, marine environments are considered to be relatively isotopically homogeneous with respect to source carbon (Fry and Sherr 1984), although spatial and temporal variations have been noted (Tamelander et al. 2012). Relative homogeneity of the Cumberland Sound marine environment could account for the low variability in  $\delta^{13}$ C and  $\delta^{15}$ N found here for anadromous fish, as well as for the isotopic similarity of anadromous fish from the two study sites.

Resident and immature fish in both systems showed considerable overlap in body size, yet the two groups were isotopically distinct. As previously introduced, immature fish were identified as the anadromous ecotype. Age at first migration for Arctic char in Arctic regions ranges from 3 to 9 years (Johnson 1980), and immature anadromous fish

have been reported to return to fresh water after only a 5 to 6 week period of feeding at sea. Therefore, resident fish may not be the only Arctic char in the lakes during the time when anadromous fish are absent, and in the event that resources were limited, immature and resident Arctic char could be competitors for those limited trophic resources. It is possible that this type of competition has led to trophic niche separation between immature and resident Arctic char. My findings support research done by Loewen et al. (2009), who compared the morphology of sympatric resident and undeveloped (i.e. immature or small-sized resting) Arctic char within these two lakes. The authors concluded that undeveloped and resident fish were distinct morphologically: resident Arctic char from these systems had significantly larger eye diameter, longer pectoral and pelvic fin lengths, and longer upper jaw lengths (for Iqalugaarjuit only) compared to undeveloped Arctic char, and therefore suggested differences in habitat use (Loewen et al. 2009). The results found in the present study showing lower carbon and nitrogen stable isotope ratios of residents compared to immature fish implies that these groups are occupying different trophic niches, with the isotopic ratios of immature fish suggesting a more marine-based carbon source and feeding at a higher trophic position. Based on age of first migration and age at maturity, Arctic char from the Cumberland Sound region begin annual marine migrations prior to becoming reproductively mature (Moore 1975a,b), which helps to account for the niche difference observed in the stable isotope ratios.

The higher  $\delta^{13}$ C exhibited by some residents from Qasigiyat, which overlap with anadromous fish, suggests that residents in this system may be feeding on marine prey in the intertidal zone, a finding first reported at this site by Loewen et al. (2009). Use of the

intertidal habitat is facilitated by the physical characteristics of this open lake system, especially during spring tides (i.e. the point in the tidal cycle where tide levels are most extreme) when there may be little to no barrier between the marine environment and the lake (Loewen et al. 2009). Furthermore, the waters at the head of Ptarmigan Fiord have low salinity (Loewen et al. 2009), which may make it physiologically possible for smallsized fish to inhabit this environment, or make use of this area for feeding. There are several possible explanations for intertidal habitat use by resident Arctic char in this system. Loewen et al. (2009) suggested adaptation due to a lack of suitable rearing habitat within the lake proper. Alternatively, by occupying the estuarine zone when physical conditions permit, residents may increase their access to trophic resources (i.e. expand their trophic niche). Knudsen et al. (2006) used this trophic niche expansion hypothesis to explain the presence of two sympatric Arctic char morphs in Fjellfrøsvatn, Norway, suggesting that a profundal morph evolved from an ancestral population consisted of littoral-feeding individuals to take advantage of an unutilized benthic feeding niche. In effect, this strategy may allow residents from Qasigiyat to alleviate intraspecific competition with immature fish of similar size. Fish density within Qasigiyat may well be the driver for resident use of the intertidal zone, as high densities of Arctic char were observed in this lake.

There were distinct differences in the Arctic char between the two study sites, as observed in the morphology of residents, as well as in the stable isotope and fatty acid profiles. Resident fish from Qasigiyat were relatively similar in colouration and morphology to anadromous fish when compared to the striking differentiation between the resident and anadromous fish from Iqalugaarjuit. A high degree of morphological

variability in landlocked Arctic char has been observed elsewhere (Gislason et al. 1999, Fraser et al. 2008), and has been noted for these lakes specifically (Loewen et al. 2009, 2010). Differences in the physical characteristics of the two lakes were also observed, and the colouration of the Arctic char seemed to mimic the colouration of the lakes: residents from clear, blue-coloured Qasigiyat were silvery, whereas residents from turbid, brown-coloured Iqalugaarjuit were dark, brown-yellow coloured, with distinct spots (reference photographs are given in Appendix E). This result is consistent with the ecological specialization of Arctic char noted throughout its distribution (for a review, see Jonsson and Jonsson 2001).

The significant size difference between residents in the two lakes was initially somewhat puzzling, because we might expect that resident Arctic char utilizing the marine environment, as the Qasigiyat residents are hypothesized to do, would have access to a more productive trophic resource, and thus be able to attain a larger body size. Yet Qasigiyat residents were significantly smaller than Iqalugaarjuit fish. This could be related to the type of prey the resident fish focus on within each system. In a study of trophic ecotypes of landlocked Arctic char, Fraser et al. (2008) found that the benthivorous fish grew larger than the planktivorous morph. Loewen et al. (2009) found a significantly longer upper jaw length in residents compared to undeveloped fish in Iqalugaarjuit, suggestive of a subterminal mouth; however, the authors found no difference in upper jaw length between these fish in Qasigiyat. The morphology of subterminal mouths suggests benthic feeding specialization (Malmquist 1992, Snorrason et al. 1994); therefore, resident Arctic char from Iqalugaarjuit may be more specialized to a benthivorous trophic niche and reach larger sizes than residents from Qasigiyat.

Analysis of stomach contents of the resident ecotype would help to further investigate resident trophic niches. Alternatively, these data imply differences in overall productivity of the two lakes, and the larger residents from Iqalugaarjuit could be the result of a more productive lake. This could provide further explanation for use of the estuarine environment by residents from Qasigiyat, as the low productivity of the lake could be forcing smaller fish into the intertidal zone to find sufficient resources. Qualitative observations of the lake colourations, vegetation, and presence of potential prey provide evidence for differences in lake productivity, thus supporting this latter hypothesis.

Although significant  $\delta^{13}$ C and  $\delta^{15}$ N differences were found between study sites, it is not possible to interpret this data with the present lack of baseline stable isotope ratios for Cumberland Sound and these study sites. Carbon ratios in consumer organisms – including fishes – can vary significantly in carbon isotope baselines due to differences in primary carbon source (Peterson and Fry 1987). Similarly, baseline nitrogen levels in the aquatic environment may vary owing to variation in terrestrial inputs, the amount of subsurface NO<sub>3</sub><sup>-</sup>, rates of N<sub>2</sub> fixation, and anthropogenic influences (Montoya 2007), although the latter is likely negligible in this region. The differences in stable isotope baselines are particularly relevant for  $\delta^{-13}$ C in freshwater systems, owing to relatively high inputs of terrestrial organic matter (Fry and Sherr 1984). Therefore the variability in baseline  $\delta^{13}$ C and  $\delta^{15}$ N between and within ecosystems can bias the interpretation of results, especially relating to trophic position (Vander Zanden et al. 1997, Post et al. 2002). Future work should include collection of a prey organism common to both systems to set these baselines (Vander Zanden et al. 1997).

The significantly higher carbon stable isotope ratio of anadromous fish between the

study years suggests a shift has occurred in the carbon isotopic baseline of the marine environment. Considering that parallel <sup>13</sup>C-enrichment was evident for the anadromous fish from both study sites, it may represent an ecosystem-wide change, encompassing the entire marine environment within Cumberland Sound. The factors affecting baseline  $\delta^{13}$ C are currently debated in the literature, but include changes in primary productivity and increases in anthropogenic CO<sub>2</sub> (Hobson et al. 2004, Tamelander et al. 2012). Long-term baseline  $\delta^{13}$ C shifts (<sup>13</sup>C-depletion) were proposed by Schell (2000) for the Bering Sea using bowhead whale (*Balaena mysticetus*) baleen, and Hobson et al. (2004) in the Gulf of Alaska with data from Steller sea lion (*Eumetopias jabatus*) teeth and seabird feathers (tufted puffins *Fratercula cirrhata* and crested auklets *Aethia cristatella*), from 50 and 25 years of data, respectively. Only two years of data are available for the present study, which does not preclude the possibility of yearly variability. A longer time-series that also incorporates prey information is required to draw conclusions from these data (e.g. Vander Zanden et al. 2003, Hobson et al. 2004).

Similarly, the nitrogen stable isotope ratio was significantly higher in 2004 and 2011, but only for Qasigiyat resident and Iqalugaarjuit anadromous Arctic char. Again, it is impossible to draw specific conclusions from these data owing to the short time series and the lack of baseline information. However, novel research has shown that other Arctic char populations in the region have experienced a shift in diet from primarily amphipod crustaceans to a newly available marine forage fish – capelin (see Section 3.3.1). The higher  $\delta^{15}$ N within Iqalugaarjuit in 2011 may have resulted from Arctic char feeding on this new diet item. Capelin represent a higher trophic level food source, as in other regions they have been reported to feed on invertebrates, primarily copepods,

amphipods, and euphausiids (Newfoundland and Labrador, O'Driscoll et al. 2001; Greenland, Hedeholm et al. 2012). The reported  $\delta^{15}N$  of capelin in Cumberland Sound has ranged widely; Dennard et al. (2009) reported capelin  $\delta^{15}N$  of 9.47 ± 0.26‰, whereas Marcoux et al. (2012) reported  $\delta^{15}N$  of 13.83 ± 0.47‰. The  $\delta^{15}N$  of amphipods in Cumberland Sound has not been documented, but in other regions the reported  $\delta^{15}N$ ranges from 5.8‰ for *Gammarus* spp. to 10.9‰ for *Onisimus* spp. (Legezynska et al. 2012). Petursdottir et al. (2012) reported stable isotope ratios for both zooplankton and capelin in the Iceland Sea. In this ecosystem, zooplankton occupied lower trophic levels than capelin (Petursdottir et al. 2012).

The resident  $\delta^{15}$ N increase (Qasigiyat) between these two years is equally as interesting, although specifics of prey assemblage or diet composition are not known. The  $\delta^{15}$ N of resident fish in this system has increased 3.09‰ between 2004 and 2011. As introduced previously, the <sup>15</sup>N-enrichment across trophic levels is usually estimated at 3-4‰ (Mingawa and Wada 1984, Hobson and Welch 1992). Gallagher and Dick (2010) found two trophic levels of Arctic char within a landlocked lake and postulated that the higher trophic level, which was 4.01‰ higher, were piscivorous and potential cannibals. Similarly, Guiguer et al. (2003) reported 3.2-3.3‰ <sup>15</sup>N-enrichment in piscivorous Arctic char over the sympatric small-form individuals that fed primarily on invertebrates. Considering these enrichment factors, the  $\delta^{15}$ N difference between the two years of this study could represent an increase of an entire trophic level, and it is possible that resident fish may have adopted a cannibalistic strategy. Cannibalism in Arctic char is common, although this strategy is usually employed by large-form individuals in landlocked Arctic char populations (Hobson and Welch 1995, Hammar 2000, Guiguer et al. 2002,

Gallagher and Dick 2010). Interestingly, a resident Arctic char was found when sampling Qasigiyat with a young-of-the-year conspecific in its mouth, providing evidence for cannibalism and/or piscivory. Sticklebacks are thought to be the only other fish species present in this system (Loewen 2008). Amundsen et al. (2000) proposed that cannibalism in Arctic char populations occurred primarily due to low productivity and lack of other food sources. This observation of cannibalism in Qasigiyat lends further support to the hypothesis that this is an unproductive lake, thereby forcing resident Arctic char to adopt alternative foraging strategies.

The combined use of stable isotope profile and fork length provides a solid basis on which to assign ecotype affinity to mature individuals. The bimodal size range of Arctic char in these systems is characteristic of partially migratory populations, in which residents have smaller size-at-maturity and maximum sizes than anadromous fish (Tallman et al. 1996). Although the use of fork length in differentiating between ecotypes is useful in field situations, the use of mature fish size to assign ecotype would be most reliable when paired with isotopic signature. Other methods have also been used to successfully identify ecotypes, such as otolith microchemistry (Swanson et al. 2010). However, the current popularity of stable isotope analysis has seen many laboratories now equipped with the appropriate equipment. Compared to other methods, SIA allows large number of samples to be analysed relatively quickly. In addition, archived tissue may be available from previous research programs, such as the samples used in this study from Fisheries and Oceans Canada stock assessment program. Other long-term studies have even made use of preserved museum specimens (e.g. Vander Zanden et al. 2003). In future, researchers may be able to employ non-lethal sampling techniques to conduct

stable isotope analysis for the purpose of ecotype discrimination, as recent work on a freshwater fish, walleye (*Sander vitreus*), has shown that fin tissue can provide similar isotopic information as muscle (Fincel et al. 2012).

The relationship between  $\delta^{15}$ N and fork length in anadromous fish found here likely reflects changes in diet as the fish grows. As reported by other researchers using stomach content analysis, these diet shifts often involve switching from invertebrate to fish-based diets with increasing size (Moore and Moore 1974, Dempson et al. 2002). In northern Labrador, smaller Arctic char (L<sub>F</sub> < 150 mm) were found to feed on invertebrates, whereas larger Arctic char began to shift towards piscivory, beginning to feed on larger fish such as capelin at L<sub>F</sub> of 300 mm (Dempson et al. 2002). This diet shift should be evident in stable isotope profiles as an increase in  $\delta^{15}$ N, because fish generally occupy higher trophic positions than invertebrates (e.g. Hobson and Welch 1992).

In contrast to anadromous Arctic char, residents showed no correlation between fork length and  $\delta^{15}$ N. No reported size- $\delta^{15}$ N relationships exist for resident Arctic char. The lack of relationship between fork length and  $\delta^{15}$ N may be due to variation in the  $\delta^{15}$ N of prey organisms from the freshwater environment, coupled with the small size range observed for the resident ecotype. There are reports of variable relationships between  $\delta^{15}$ N and fork length in salmonids. Power et al. (2002) reported an inverse correlation between  $\delta^{15}$ N and fork length in a landlocked population of Arctic char, and suggested that larger prey items do not necessarily have higher  $\delta^{15}$ N than smaller prey items. Similarly, Vander Zanden et al. (2000) concluded that the lack of a relationship between trophic position and size for prey species helped describe the same phenomenon in predatory lake trout. L'Abee-Lund et al. (1993) point out that larger juvenile Arctic char

were found in habitats that would provide the greatest foraging gain, but that greater prey density was more important than prey size.

The correlation of resident  $\delta^{13}$ C and  $L_F$  suggests size-specific use of marine derived trophic resources. Larger residents may be using more diet items from the marine environment than smaller fish, resulting in higher  $\delta^{13}$ C. The ability of Arctic char to osmoregulate in salt water has been shown to be related to size (Arnesen et al. 1992, Halvorsen et al. 1993). Although Moore (1975b) reported that Arctic char from two river systems in the Cumberland Sound region less than 100 mm never entered salt water, there is considerable variation in the size of first migration to salt water (Johnson 1980). Mature anadromous Arctic char, on the other hand, seem to feed in the same habitats regardless of body size, as indicated by no change in  $\delta^{13}$ C with L<sub>F</sub>. Investigation of baseline isotope levels is needed to further disentangle these relationships.

This study reports the first fatty acid data for sympatric resident and anadromous Arctic char, to the best of my knowledge. There has been much research on the dietary requirements, composition, and metabolism of lipids in Arctic char owing to its importance in the aquaculture industry (reviews: Ringø and Olsen 1987, Dick and Yang 2002). The fatty acids identified in the highest proportions – namely C16:0, C16:1n7, C18:1n9, DHA and EPA – are consistent with previous results for wild Arctic char (Ringø and Nilsen 1987, Yang 1994), as well as other marine and freshwater salmonids (Chinook and coho salmon, Daly et al. 2010; Atlantic salmon, Budge et al. 2012).

Resident and anadromous Arctic char exhibited different fatty acid signatures, which supports the trophic niche differences indicated by stable isotope analysis, as discussed above. The differences between ecotypes were observed in a suite of fatty acids; principal components analysis indicated that no single fatty acid was responsible for the differences between Arctic char ecotypes. Fatty acid composition is strongly influenced by diet (review: Dalsgaard et al. 2003), which has been shown for Arctic char with experimental studies comparing the fatty acid composition of tissue of fish under different diet regimes. Olsen et al. (1999) experimentally fed two groups of Arctic char diets with different levels of polyunsaturated fatty acids (PUFA), and found that the fatty acid composition of muscle tissue was significantly different between the groups, although fatty acid modification was also observed. Studies of wild fish have also shown this relationship: Heissenberger et al. (2010) found significant differences in the fatty acid profiles of lake-dwelling and aquaculture-raised Arctic char that also differed in diet.

Some general patterns of fatty acid differences between freshwater and marine fish have been previously described (Gruger et al. 1964, Ackman 1987, Henderson and Tocher 1987). Despite being classified as a freshwater fish (Johnson 1980) and having a saltwater residency spanning a maximum of four months in this region (Moore 1975a), the adult anadromous Arctic char can still be considered marine (like other anadromous salmonids; Henderson and Tocher 1987), especially considering their reliance on marine trophic resources (Dempson et al. 2002, see Section 3.3.1). Freshwater fish generally have higher C18 polyunsaturated fatty acids, particularly LIN and ALA (Ackman 1987), lower EPA (Gruger et al. 1964) and DHA, and higher ARA than marine fish (Özogul et al. 2007). Stansby (1967) noted fatty acid differences between freshwater and marine coho salmon, specifically that the freshwater ecotype had a much higher proportion of linoleic acid (LIN; C18:2n6), and much lower proportion of EPA than the sea run ecotype, also noting a slightly lower proportion of DHA in freshwater residents. My

findings are consistent with the former two observations, as residents had higher LIN and lower EPA than anadromous fish. DHA is commonly asserted to be lower in freshwater organisms than marine (Dick and Yang 1994). It is somewhat surprising that anadromous fish had significantly lower proportions of DHA than residents; yet the DHA proportions I found for anadromous and resident fish closely resemble those reported for wild Atlantic salmon and freshwater rainbow trout, respectively (Blanchet et al. 2005). The DHA proportions for resident and immature fish are consistent with values reported for juvenile Chinook and coho salmon (Daly et al. 2010). Similarly, my results are congruent with the multi-species study done by Özogul et al. (2007), who concluded that in general, freshwater fish exhibit higher ARA.

Given the importance of diet in Arctic char fatty acid composition, it is important to consider the fatty acid composition of potential prey items to understand the differences between the resident and anadromous fish observed here. McMeans et al. (2012) recently reported the fatty acid profile of the herbivorous marine copepod (*Calanus hyperboreus*) within the Cumberland Sound system and, like copepods in other systems (Southern Ocean, Connan et al. 2010; Arctic Ocean, Lee 1975), this species was found to be rich in monounsaturated fatty acids, particularly C16:1n9, C20:1n7, and C20:1n11. Given this information, the significantly higher proportion of MUFAs in anadromous fish compared to residents within both study sites suggests that copepods have a trophic importance for Arctic char, either directly (as a diet item) or indirectly (as prey for diet items). In addition, the summer DHA proportions for this copepod species were reported between  $8.9 \pm 0.4$  and  $9.3 \pm 0.4$  (McMeans et al. 2012), which could explain the lower DHA proportion for anadromous fish compared to residents if these copepods are an integral

component of the food web. The fatty acid signatures indicative of feeding on *C*. *hyperboreus* have also been found in pelagic amphipods *Themisto* spp., and *Gammarus* spp. in the Iceland Sea (Petursdottir et al. 2012). There is evidence that these zooplankton (copepods, *Themisto* spp., and *Gammarus* spp.) are included in the diet of anadromous Arctic char from other lake systems within Cumberland Sound (see Chapter 3), as well as from other regions of the Canadian Arctic (Dempson et al. 2002).

The difference between the fatty acid profiles of the two ecotypes was somewhat more distinct in Iqalugaarjuit than Qasigiyat, supporting stable isotope results, but was much less pronounced in the fatty acid profiles. However, there were no specific indications in the fatty acid profiles that residents from Iqalugaarjuit were feeding on marine prey.

Mirroring the results of the nitrogen stable isotope analysis, immature Arctic char from Iqalugaarjuit were markedly different from both resident and anadromous ecotypes in their fatty acid profiles. Differences in the fatty acid compositions of mature and juvenile coho salmon were noted by Stansby (1967), in which C18:2 was higher, and C20:5 and C22:6 were lower in juvenile salmon. Similarly, Henderson and Tocher (1987) stated that salmonid smolts had fatty acid compositions more similar to marine fish than fresh water fish. The lack of difference in individual fatty acid proportions between Qasigiyat resident and immature fish seems to indicate close similarity between these two groups; however, multivariate analysis revealed that immature fish had a large degree of variability in their fatty acid profiles, with some individuals more similar to residents and others resembling anadromous fish. The disparity between the interpretations of these results highlights the importance of using a multivariate approach to analyse fatty acid data. My results suggest that whereas Iqalugaarjuit immature fish have little overlap in trophic niche with adults of either ecotype, immature fish from Qasigiyat adopt either a marine or fresh water based feeding strategy and as a group do not necessarily occupy a distinct trophic niche. It is likely that in this system the smaller immature fish have more trophic overlap with resident fish, remaining within fresh water and/or the intertidal zone, whereas larger immature fish are migrating to the marine environment with their adult anadromous counterparts. Anadromous Arctic char generally make multiple migrations prior to becoming sexually mature (Johnson 1980, Gulseth and Nilssen 2000). The age at first maturity for anadromous fish in these systems has been reported to range between 7-17 years (Qasigiyat) and 4-17 years (Iqalugaarjuit) (Loewen et al. 2010). Although the age at first migration has not been documented for these sites, it can range from 2 to 9 years (Johnson 1980), and is documented as averaging 5 years in nearby lake systems (Moore 1975a). From the results of stable isotope analysis, it is apparent that most of the individuals within the Iqalugaarjuit study site are migrating to salt water to feed. Yet the differences between the migrating immature and mature anadromous fish suggest that perhaps immature fish do not venture as far from the river mouth, which is consistent with the findings of Moore (1975), who determined that fish 6-9 years ( $L_F = 10-20$  cm) did not travel as far as older/larger fish.

Individual fish with ambiguous morphology were not easily classified in some cases, despite the combined use of stable isotope and fatty acid analysis. In Qasigiyat, the ambiguous fish appeared to be immature, and immature fish exhibited a wide range of both stable isotope and fatty acid profiles, overlapping both resident and anadromous ecotypes. As per the previous discussion, the ambiguous fish that grouped with the anadromous ecotype were likely immature fish using a migratory strategy, whereas the ones that grouped with residents likely used more fresh water and/or estuarine trophic resources. In Iqalugaarjuit, one of the ambiguous fish appeared to be a resident, and it also appeared to be immature. Fish with ambiguous morphology may be immature fish of either ecotype, as ecotype-specific morphology may not yet be developed. Previous experimental work has shown that polymorphism related to resource use can be observed in young-of-the-year and 1 year old Arctic char (Andersson et al. 2005). It is not known at what age or size ecotype differentiation occurs within these populations.

The fatty acid profiles of the two mature ambiguous fish truly warrant the label ambiguous, as these Arctic char exhibited resident-like stable isotope profiles, but seemed to be different from both resident and especially anadromous fish in fatty acid profiles. These results suggest that these fish remained in fresh water over the previous feeding season, and have experienced a morphological change, because they could not be visually placed in a specific category. Arctic char have a high degree of phenotypic plasticity, as shown by morphological changes in  $\leq$  1-year old individuals after only 55 days in a resource use experiment (Andersson et al. 2005). In addition, the large number of sticklebacks and chironomid larvae observed within the Iqalugaarjuit lake indicated that there may be sufficient resources to support larger fish.

In conclusion, this study presents data to confirm the use of stable isotope analysis as a valid tool to discriminate between sympatric anadromous and resident Arctic char. My hypothesis that resident and anadromous Arctic char would have distinct trophic niches has been supported, as shown through markedly different stable isotope and fatty acid profiles. As expected, the trophic niche of anadromous Arctic char was based in the

marine environment, and the niche of resident Arctic char was in the fresh water environment. In some cases, however, resident fish seem to be capable of utilizing the estuarine environment for trophic resource use, as supported by the data presented here. In addition, immature Arctic char seem to occupy a distinct niche from both anadromous and resident fish, which likely relates to use of both freshwater and estuarine environments, depending on developmental stage. Analyses of available diet items and stomach contents of resident and juvenile Arctic char would help to further investigate the trophic relationships within this system.

# **Chapter 3** The capelin invasion: evidence for a trophic shift in Arctic char populations from the Cumberland Sound region

# Abstract

Climate change is having myriad effects on Arctic marine ecosystems and food webs. In the Cumberland Sound region of the Canadian Arctic, anadromous Arctic char rely on an intensive summer feeding season at sea, and there has been a recent invation of a forage fish, capelin, into this ecosystem. To investigate possible changes over time, two populations of Arctic char from this region were sampled for biological data, stomachs, and muscle tissue from 2002 to 2011. I assessed Arctic char trophic niche using stomach content analysis and stable isotope analysis ( $\delta^{13}$ C and  $\delta^{15}$ N) of muscle tissue, and population characteristics by calculating Fulton's condition factor (K) and by fitting von Bertalanffy growth curves with length-at-age data. Results revealed capelin newly present in the diets of Arctic char in 2011, describing a shift from a primarily invertebrate-based to a fish-based diet within the past decade. Lack of a significant trend in  $\delta^{15}$ N over time suggests the trophic level of Arctic char has not changed; however, the  $\delta^{15}$ C for both systems converges in 2011 on a value suggestive of feeding on capelin. Results must be interpreted with caution in the absence of baseline isotope data. Condition (K) was variable throughout the study period, but did not show a significant directional change. Although not conclusive, comparisons of von Bertalanffy parameters suggest that foraging on capelin may be positively affecting the growth of these Arctic populations. The diet shift documented here may provide the first evidence of a more widespread change in the marine ecosystem of Cumberland Sound affecting other marine species in this Arctic food web. In addition, changes in the growth and condition of Arctic char in this region could have significant economic and cultural implications.

## **3.1 Introduction**

Describing the trophic niches of species within Arctic systems is particularly relevant, as there is currently much concern over anthropogenic impacts on Arctic ecosystems, including the effects of climate changes and natural resource exploitation (Schofield et al. 2010, Portner et al. 2010). Climate change is having a considerable impact on Arctic environments, leading to altered productivity, species distributions, and food web dynamics, in addition to numerous abiotic properties (van Hal et al. 2010, Schofield et al. 2010). These changes can have species-level effects that, in turn, can influence the sustainability of resource exploitation, of which fisheries is a prime example (Brown et al. 2010). For instance, changes in sea surface temperature around Iceland has been cited as a probable factor in changing fish species distribution in the region, including range expansions and contractions, and more frequent occurrences of transient species (Valdimarrson et al. 2012). As a result, it has become increasingly important to investigate potential changes within a species' trophic niche and the effects of these shifts on populations to allow appropriate management of these systems.

Since the early 2000s, capelin have been observed inhabiting the Cumberland Sound ecosystem in Nunavut, Canada. Local ecological knowledge and previous marine research activities conducted in this region suggests that this small planktivorous fish was not present in the region prior to this time (J. Sowdluapik, pers. comm., Moore and Moore 1974). Capelin populations have historically undergone extensive distribution shifts that have been linked to the species' ability to respond quickly to changes in ocean temperature, which led Rose (2005) to call the species a "canary" of the sea with respect to climate changes. Changes in capelin abundance and distribution have been noted in

other regions in recent years (Dempson et al. 2002, Gaston et al. 2003), including in the Iceland Sea, where recent changes in capelin distribution have been attributed to increases in water temperature of 1-2°C (summer surface temperature) since the mid-1990s (Valdimarsson et al. 2012). These changes support the suggestion of Huse and Ellingsen (2008) that capelin would establish new spawning areas in response to predicted climate change scenarios. This pelagic fish species represents a new, potentially high-quality forage for higher trophic level predators within the Cumberland Sound ecosystem; therefore, it is likely that its presence will have wide-ranging impacts on this Arctic marine food web.

Anadromous Arctic char undertake an annual migration to salt water for an intensive summer feeding period lasting from 30 to 50 days (Finstad and Heggberget 1993, Jørgensen et al. 1997), during which they can increase their lipid stores up to fivefold (Jørgensen et al. 1997). These fish are obligated to return to fresh water every year for overwintering and/or spawning, where food intake is low or absent (Johnson 1980). In this way, Arctic char are able to take advantage of the relatively high productivity of the marine environment, a benefit that is hypothesized to be the main driver of anadromy (Gross et al. 1988). The foraging strategy of Arctic char in the marine environment is characterized as opportunistic at the population level and variable at both a spatial and temporal scale, to a large extent reflecting prey density and availability (Amundsen 1995, Dempson et al. 2002, Rikardsen et al. 2007). In contrast to the much-studied feeding ecology of landlocked Arctic char (e.g. Saksgård and Hesthagen 2004, Amundsen et al. 2008, Fraser et al. 2008), the marine diet of anadromous Arctic char is relatively poorly documented, although Dempson et al. (2002) and Rikardsen et al. (2007) described the

marine diets of char in Labrador and Norway, respectively, using stomach content data.

Arctic char diet composition during the marine feeding period has been hypothesized to reflect prey availability, although fish size is also a factor (Rikardsen et al. 2007). Pelagic invertebrates have been reported to make up a large component of Arctic char diets. These include amphipods (*Themisto* spp., *Gammarus* spp., and others), krill (*Thysanoessa* spp.), and shrimp (*Mysis* spp.) (Moore and Moore 1974, Dempson et al. 2002, Rikardsen et al. 2007). In some regions, fish make up a significant component of the diet, including species such as herring, gadoids, sand lance, and notably, capelin (Dempson et al. 2002, Rikardsen et al. 2007).

A previous diet study of anadromous Arctic char indicates that Cumberland Sound populations historically relied on invertebrate prey, particularly amphipods (Moore and Moore 1974). However, this may no longer be the case, as preliminary data shows that some Arctic char in the Cumberland Sound region may be incorporating capelin into their diet (R. Tallman, unpubl. data). Capelin is known to be an important forage species for Arctic char in regions where ranges of these two species overlap, such as Labrador, Canada (Dempson et al. 2002). In Labrador, drastic changes in capelin availability have been linked to shifts in Arctic char diet and changes in Arctic char population characteristics (Dempson et al. 2002, 2008). More specifically, Arctic char growth rates decreased during a period of anomalous climate conditions that was accompanied by significant decreases in capelin availability (Michaud et al. 2010). Capelin are considered to be a relatively fatty fish, with a percent lipid content ranging from 3 to 8% for the months they would potentially be available to Arctic char (Montevecchi and Piatt 1984, Huynh and Kitts 2009). Lawson et al. (1998) compared north Pacific fish prey species, and concluded that capelin had the highest energy density, thus prey quality. Energy densities for capelin from West Greenland were reported by Hedeholm et al. (2012) as ranging between 20.86 - 24.80 kJ per gram of dry weight, similar to reported values for other high quality prey items such as copepods from the same region (Hedeholm et al. 2012). The apparently high quality of capelin as prey and the observations of local fishermen in the community of Pangnirtung (J. Sowdluapik, pers. comm.) led me to suspect that Arctic char were feeding extensively on capelin and hypothesize that the Cumberland Sound Arctic char populations may be benefiting – in terms of growth and condition – from this new food source.

Descriptions of trophic niche in fish are generally accomplished using dietary analysis. Fish diets are often studied through analysis of stomach contents (Moore and Moore 1974, Dempson et al. 2002), which allows the researcher to identify and quantify individual prey items (Cortés 1997). However, stomach contents only provide information on the diet of an individual fish within a short period of time immediately prior to capture. In species with an opportunistic and highly variable feeding strategy – such as Arctic char – a snapshot view of diet may not fully capture feeding patterns or trends within a population. Stomach content analysis may also cause biases owing to differential digestion and subsequent underrepresentation of soft-bodied organisms and may pose substantial difficulties in prey identification (Iverson et al. 2004). In addition, logistical constraints may require sampling at a time when fish stomachs are empty (Adams et al. 2003) or using methods that cause evacuation of gut contents (Stowasser et al. 2009), precluding the use of stomach content analysis.

To overcome some of the constraints of traditional stomach content analysis,

biochemical techniques have been widely adopted for trophic investigations (Post et al. 2002, Iverson et al. 2004). These techniques are based on the premise that certain components of the prey (biomarkers) are incorporated into the body tissues of the predator, and therefore are an indication of the predator's assimilated diet (DeNiro and Epstein 1978, Mingawa and Wada 1984, Iverson et al. 2004). Biomarkers such as stable isotopes have the capacity to create a comprehensive picture of diet over the long term, from weeks to months (Bootsma et al. 1992, Wan et al. 2010). Stable isotopes, particularly of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N), have proven to be useful tools for elucidating long-term food web changes and diet shifts (Hebert et al. 2002, Schmidt et al. 2009). Long-term food web changes have been suggested for the North Pacific and Gulf of Alaska (Hobson et al. 2004), the North Sea (Christensen and Richardson 2008), and Cumberland Sound (Marcoux et al. 2012) based on stable isotopes. This technique has also been used to document diet shifts in fish in response to species invasions, such as the research of Eagles-Smith et al. (2008) that assessed the impact of an invasive fish, threadfin shad (Dorosoma petenense), on the diet of native fish species in Clear Lake, California, USA. However, stable isotopes are limited to inferrences about actual diet items, information that stomach contents can provide, and unless specifically validated, researchers must make numerous assumptions when drawing conclusions using stable isotope profiles, particularly for assessing trophic position, estimating diet source, or gauging change over time (Vander Zanden et al. 1997, Post et al. 2007). These assumptions include baseline stable isotope ratios, tissue-diet discrimination factors, and isotope turnover rates in tissues (Vander Zanden et al. 1997, Trueman et al. 2005, Post et al. 2007).

This study was undertaken to describe the marine diet of Arctic char in the Cumberland Sound region and assess its stability throughout the last decade. Specifically, I hypothesized that capelin would be a dominant diet item for Arctic char in 2011, but would be absent in the early 2000s. I expected this shift to be evident both in stomach contents and nitrogen stable isotope ratios of these Arctic char populations over this period. Given the importance of the brief marine feeding period in the life cycle of anadromous Arctic char and the possible benefits obtained by feeding on this apparently high quality diet item, I also assessed population parameters (condition and length-at-age) to explore the possible effects of a diet change.

#### **3.2 Materials and Methods**

## 3.2.1 Study Sites

Cumberland Sound is an extension of the North Atlantic in the Canadian Arctic, extending northwest from Davis Strait and bordered by the fiords of southeast Baffin Island (Fig. 12). According to the Canadian Hydrographic Service, the marine environment in this region is characterized by extreme tides, with tidal cycles exceeding 7.2 m above and 0.3 m below chart datum (CHS 2011).

This study focused on two lake systems with freshwater lake basins connected to the marine environment by streams passable by Arctic char: Isuituq (PG080), and Kipisa (PG004) (Fig. 12).

Isuituq (66°63'N, 67°92'W) is located at the head of Cumberland Sound, the outflow forming the head of Clearwater Fiord. This system encompasses the estuarine environment of the Isuituq River, where the outflows of multiple open lake systems converge (Harris and Tallman 2010; Fig. 13). The Arctic char sampled at this location

likely represent populations originating from several of the surrounding lakes (Harris and Tallman 2010). At low tide, a long (approximately 1 km) tidal flat is exposed, revealing a sandy substrate strewn with boulders and with braided channels of outflowing salt water mixed with fresh water from the river (Fig. 14a). At high tide, the saltwater completely covers the flat to the mouth of the river (Fig. 14b).

Kipisa (66°52'N, 67°85'W), at the tip of Kangilo Fiord, is located at the head of Cumberland Sound (Fig. 12). The tidal flat in this system is less extensive than Isuituq, with a single channel extending along the flat through the estuarine zone at low tide (Fig. 15). The substrate is sandy with boulders.

Both lakes are currently designated as exploratory fishing waterbodies (i.e. low levels of commercial fishing are permitted), and in addition to this fishing quota, local indigenous people fish these areas for subsistence purposes. Detailed stock assessments have been conducted by Fisheries and Oceans Canada (DFO 2005, 2010), which have concluded that exploratory, subsistence, and experimental fishing activities have had minimal impact on the health of the Arctic char population within each system. The study systems were chosen for this research because of archived data availability from these stock assessments, as well as consistency in the timing (i.e. month) of data collection each year, because diet and stable isotope signatures are often seasonally variable in salmonids (Doucett et al. 1999*a*, Dempson et al. 2002, Ciancio et al. 2008).



**Figure 12.** Location of the Kipisa (PG004) and Isuituq (PG080) study sites in the Cumberland Sound region, showing the community of Pangnirtung, Nunavut. Inset map shows the location of Cumberland Sound along southeast Baffin Island in the Canadian Arctic.



Figure 13. Map of the Kipisa (PG004) and Isuituq (PG080) study sites, showing locations of sampling.



**Figure 14.** The Isuituq study site in July 2011 at (a) low tide and (b) high tide. Arctic char were captured in the marine environment at high tide.

# 3.2.2 Field Collections

Arctic char were sampled during the summer of 2011, from August 15 to 17 (Isuituq) and from August 23 to 25 (Kipisa). To maintain consistency with the archived data, sampling followed standard DFO stock assessment protocol, as follows. Multi-mesh gillnets with stretched mesh sizes ranging from 38.1 to 139.7 mm (DFO 2010) were set perpendicular to the shoreline in the intertidal zone between the saltwater fiord and the freshwater river at depths of 0.5 to 7 m (at high tide). Nets were positioned to intercept the anadromous fish as they began to migrate toward the rivers to the spawning/overwintering lakes. Nets were checked regularly and all captured fish were removed for sampling. Fishing was concluded when a representative sample of the population (approximately 200 fish; VanGerwen-Toyne and Tallman 2011) had been captured (Isuituq, n = 201; Kipisa, n = 197).



**Figure 15.** Local fishermen checking gillnets at the Kipisa study site in August 2011. Fish were captured in brackish water between the fiord and the river.

Each captured fish was sampled for fork length (mm), round (i.e. whole) weight (g), sex, and maturity status (mature, immature, resting, ripe, or spent) (Gillman and Sparling 1985). Sagittal otoliths were removed, cleaned, and stored in a dry envelope. A plug of white muscle (approximately 2 x 5 cm) was cut from the dorsal surface of each fish, from a landmarked location immediately posterior to the head and anterior to the dorsal fin

(Fagan et al. 2011). The digestive tract was removed from each fish for later stomach content analysis in the laboratory. Stomachs and tissue samples were placed in airtight bags, labelled, and stored on ice. All samples were frozen at -20°C as soon as logistics permitted, typically 3-5 days.

### 3.2.3 Archived Samples and Data

In addition to the samples collected in 2011, I analysed archived Arctic char muscle tissue and stomach samples from the study systems that were collected during the Fisheries and Oceans Canada stock assessment sampling program in 2002, 2003, 2004, and 2008 (Table 5). The samples had been stored frozen (-20°C) in airtight bags.

Biological data (i.e. fork length, weight, age, and maturity) were available for multiple years spanning from 1998 to 2005 (Kipisa) and 2002 to 2008 (Isuituq) (Table 5). Although a resident (i.e. non-migratory) ecotype is known to exist in both systems, all archived samples were assumed to be anadromous, because sampling was conducted in the marine environment (DFO 2005, 2010).

To control for possible ontogenetic differences among individuals and years (Forseth et al. 1994, Eloranta et al. 2010), only fish that had reached sexual maturity (i.e. those with a maturity status of mature, ripe, resting, or spent) were included in the analyses. Moreover, samples for which the maturity stage of the individual was ambiguous or unknown were excluded from the analyses.

From the 2002 data for Isuituq and Kipisa, fork length and weight were provided from frozen specimens in contrast to the fresh fork length and weight data provided for all other samples. To allow comparisons among years, these data were converted into fresh fork length and fresh weight, respectively, using the following conversion factors for Isuituq (Eq. 3 and Eq. 4) and Kipisa (Eq. 5 and Eq. 6). These formulae were derived from regression analysis of the known fresh and frozen fork lengths and weights of individuals captured at their respective lakes in 2003 and 2004.

Eq. 3.	$L_{FFresh} = 1.025  \ast  L_{FFrozen} + 1.535$
Eq. 4.	$W_{Fresh} = 0.988 * W_{Frozen} - 1.195$
Eq. 5.	$L_{F Fresh} = 0.964 * L_{F Frozen} + 39.864$
Eq. 6.	$W_{Fresh} = 0.983 * W_{Frozen} + 71.46$

**Table 5.** Sample sizes (*n*) of anadromous Arctic char that were analysed for biological data (i.e. fork length, weight, age, and maturity), stable isotope analysis, and stomach contents for each of two study sites in the Cumberland Sound region. Sampling was conducted with gillnets during routine stock assessment activities in July and August from 1998 to 2011. Immature fish were excluded from stable isotope and stomach content analyses.

		Biological Data		Stable	
Study	Collection		Excluding	Isotope	Stomachs
Site	Year	All	Immature	Analysis	With Prey
Kipisa	1998	153	34		
	1999	196	54		
	2002	50	30	30	27
	2003	134	127	21	14
	2004	160	120	20	18
	2011	194	84	84	25
Isuituq	2002	46	28	42	33
	2003	214	168	38	14
	2004	179	155	21	11
	2005	206	157		
	2006	194	144		
	2008	176	161	46	
	2011	200	102	102	43

## 3.2.4 Laboratory Analysis

# 3.2.4.1 Stomach Contents

Archived and freshly collected stomachs were analysed in the laboratory (Table 5). Frozen digestive tracts were partially thawed and stomachs were cut from the oesophagus to the pyloric sphincter. Stomach contents were emptied into a petri dish, and prey items were identified to the closest taxon possible. Prey were counted based on identifiable heads or hard parts (e.g. amphipod carapaces), and were then placed in a dish, blotted to remove excess water, and weighed to the closest 0.1 g. Reference photographs of each taxon were taken with a Micron camera mounted to a dissecting microscope. Representative specimens of prey items were separated, rinsed, and placed in 95% ethanol in labelled scintillation vials for reference and identification.

# 3.2.4.2 Stable Isotopes

Muscle  $\delta^{13}$ C and  $\delta^{15}$ N were analysed for each year that samples were available (Table 5). Approximately 3 g of muscle was subsampled from each frozen tissue sample and freeze-dried for a minimum of 48 hours (McMeans et al. 2010). The tissue was subsequently ground to a fine powder using a mortar and pestle and stored in a cryogenic vial. The high fat content of some samples prevented full homogenization; therefore, these samples were re-homogenized after lipid extraction. Lipids are a potentially confounding factor that must be accounted for when interpreting stable isotope analysis results. Because lipids have lower  $\delta^{13}$ C relative to other tissue constituents, such as carbohydrates, the lipid quantity and variability among samples have the potential to bias the results of carbon isotopic analyses (Post et al. 2007). The carbon to nitrogen ratios (C:N) of bulk samples have been used as predictors of lipid levels, thus, high C:N (< 4.0)
indicate high levels of lipid content, necessitating the treatment of lipids. Lipid extraction is preferred over *post hoc* mathematical normalization, especially when there is limited species-specific validation of the normalization equations (Fagan et al. 2011, see Section 4.4). However, it has been suggested that lipid extraction can lead to fractionation of the nitrogen isotope of 0.25 to 1.6‰ (Post et al. 2007, Mintenbeck et al. 2008). Initially, a subsample was analysed in bulk form to determine if lipid content (using C:N as a proxy) was high enough to warrant lipid extraction. A large proportion of the samples had C:N ratios  $\geq$  4.0, which generally indicates high lipid levels.. To avoid the aforementioned lipid effects,  $\delta^{15}$ N analysis was conducted on bulk samples (i.e. not lipid extracted) and  $\delta^{13}$ C analysis on lipid extracted samples (Sotiropolous et al. 2006). The effects of lipids and lipid extraction on the isotopic profiles of Arctic char muscle tissue are addressed in Chapter 4 Section of this thesis.

All samples were lipid extracted prior to carbon isotopic analysis using a modified Bligh and Dyer (1959) method, as follows. Approximately 4 ml of 2:1 chloroform methanol (CHCl<sub>3</sub>:MeOH) solution was added to 0.5 ml of ground tissue. The sample was then vortexed for 15 seconds and placed in a 30°C water bath. After 24 hours, the sample was centrifuged for 6 minutes, the supernatant was decanted and discarded, and another 4 ml of 2:1 CHCl<sub>3</sub>:MeOH was added. Again the sample was vortexed, centrifuged, and the supernatant discarded. The remaining solids were left to dry for 48 hours in a fume hood.

Both bulk and fully dried lipid extracted samples were individually weighed (400-600 µg) into 0.5 mg tin capsules. Analyses of carbon and nitrogen isotopes were conducted with a Delta V Advantage continuous-flow isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010 Elemental Combustion

System (Costech Instruments, Valencia, CA, USA) at the Great Lakes Institute of Environmental Research (University of Windsor, Ontario). Every thirteenth sample was run in triplicate, and four internal laboratory standards and one National Institute of Standards and Technology (NIST) reference standard were run after every 15 samples for quantification. During this analysis, analytical precision for  $\delta^{15}$ N, based on the standard deviation of a lab standard (fish muscle) and NIST standard 8414 (bovine liver), was 0.15 and 0.14‰, respectively, and for  $\delta^{13}$ C was 0.14 and 0.10‰, respectively.

In accordance with standard practices, stable isotope ratios are given as the difference in the isotopic ratio ( $\delta^{13}$ C or  $\delta^{15}$ N) between the sample and reference standards (Peterson and Fry 1987). The ratios are expressed as parts per thousand (‰) following the formula:

Eq. 1. 
$$\delta^{13}C, \ \delta^{15}N, or \ \delta^{34}S = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000$$

where R = the ratio of carbon (<sup>13</sup>C: <sup>12</sup>C) or nitrogen (<sup>15</sup>N: <sup>14</sup>N) isotopes of the sample. By convention, standards are set at 0‰.

#### 3.2.4.3 Age Estimation with Otoliths

Ages were estimated for each fish by counting the annuli of whole or embedded and sectioned sagittal otoliths (Chilton and Beamish 1982). All otoliths were initially read whole. Preliminary otolith readings that estimated an age over 10 years or that were inhibited by otolith irregularities (e.g. opacity or crystalline deposits) were embedded and sectioned. Clean, dry otoliths were placed in a strip of mixed epoxy resin with the sulcus facing up. A probe was used to eliminate air around the otolith. The epoxy was allowed to harden for 7 days. Once set, a sectioning plane was marked on the epoxy with a fine tip marker using a dissecting scope with a micrometer eyepiece. Marking ensures the sectioning line runs through the nucleus and captures the most distinct, least flawed portion of the dorsal surface of the otolith, which was usually slightly posterior to a line perpendicular to the sulcus. Otoliths were sectioned using a Buehler Isomet saw and then polished with a series of lapping films. Reading – or counting of the otolith annuli – was done with a dissecting microscope, and a confidence level was assigned to each age (R.Wastle, pers. comm.). Ages with low confidence were re-read by a second reader to confirm accuracy (i.e. that both readers read the same age); discrepancies were settled between readers, and quality control procedures were applied where the second reader reread a random selection of otoliths (R. Wastle, pers. comm.).

Ages had been previously assigned to the archived otoliths. Age estimates based on otolith annuli counts can be subject to a substantial level of error in accuracy and precision, especially among age-readers depending on experience and skill (Campana 2001). Therefore, ages were all assigned by the same reader to ensure consistency. Additionally, ages were estimated using comparable methods of otolith preparation (R.Wastle, unpubl. data). Estimated ages were used to derive length-at-age data for growth curve estimations.

#### 3.2.5 Data Analysis

Statistical analyses were conducted using SigmaStat v. 3.5 (Systat Software Inc. 2006), except where otherwise indicated. Significance was set at 0.05. Data were transformed as required to meet assumptions of normality and homoscedasticity, and when assumptions could not be met, the non-parametric test equivalent was used.

The length frequency distributions of Arctic char captured in each year were compared, because the diet of Arctic char has been shown to vary with fork length (Moore and Moore 1974, Dempson et al. 2002, Rikardsen et al. 2007). One-way ANOVAs were used to test for significant differences in mean fork length among sites and among years. The relationship between fork length and stable isotope ratios was assessed with linear regression, and upon finding significant results, fork length was included as a covariate in subsequent analyses.

# 3.2.5.1 Stomach Contents

A suite of three common diet indices were calculated to investigate the hypothesis that capelin have become a prey item for Arctic char in these systems and to detect differences in diet among years. These included frequency of occurrence (%O), which was the percentage of prey-containing stomachs in a given year that a particular prey type was found, as well as two abundance calculations: percentage by weight (%W) and percentage by numbers (%N) (Ricker 1971, Cortes 1997). Percentage by weight and numbers were calculated using the abundance (weight or number) of a particular prey item compared to the total abundance (weight or number) of all prey in the preycontaining stomachs.

As the potential Arctic char diet items may differ significantly in size, the relative importance of each main diet item category was assessed with the Index of Relative Importance (IRI), which was calculated using Eq. 7.

Eq. 7. IRI = %O \* (%N + %W)

The IRI of each diet category was converted to a percentage of the total (Grimaldo et al.

2009) and displayed graphically to facilitate identification of changes in the relative importance of different prey items over the study period. This compound index is less susceptible to differences in prey size that can skew the relative importance of other indices (Liao et al. 2001), which is important to consider in species such as Arctic char that feeds on prey varying widely in size. An exploratory assessment of the prey accumulation curves (i.e. randomly pooled stomachs plotted against cumulative number of different prey) suggested that sample sizes were sufficient to describe the diet of Arctic char during these sampling periods (Cortés 1997).

# 3.2.5.2 Stable Isotopes

Due to preliminary data showing a correlation of  $\delta^{15}$ N with fork length (Kipisa: r = 0.40; Isuituq: r = 0.46), statistical comparisons of  $\delta^{15}$ N among years were done using ANCOVA with fork length as a covariate in R (R Core Development Team 2008; see Appendix A for coding). Differences in the carbon isotope ratios were compared among years using ANOVAs, as  $\delta^{13}$ C did not correlate with L<sub>F</sub>. Pairwise comparisons were conducted with a *post hoc* Tukey's HSD test. The stable isotope ratios ( $\delta^{13}$ C and  $\delta^{15}$ N) for each year were plotted for visual interpretation of changes over time. Baseline levels of  $\delta^{13}$ C and  $\delta^{15}$ N can be variable between and within ecosystems, introducing potential bias when interpreting trophic positions and comparing among systems (Vander Zanden et al. 1997, Post et al. 2002). Because determining absolute trophic position or comparing the trophic levels of Arctic char among locations are not within the scope of this research, estimations of baseline  $\delta^{13}$ C and  $\delta^{15}$ N values were not conducted. However, baseline isotopic levels may also change temporally (Hobson et al. 2004); therefore, in the absence of baseline prev data for these systems,  $\delta^{13}$ C and  $\delta^{15}$ N for Arctic char were

compared with other consumers feeding in the marine environment during the same period to help account for the possibility of a shifting baseline.

#### 3.2.5.3 Condition

Length-weight relationships were assessed as a measure of condition, using Eq. 8, where W is the fresh weight (g),  $L_F$  is the fork length (mm), and *a* and *b* are constants (Ricker 1975).

# **Eq. 8.** $W = aL^b$

The value of b generally ranges from 2 to 4, with a value of 3 representing the classical equation for Fulton's condition factor (K; Ricker 1975). When b = 3, the fish is increasing in weight in proportion to its length; b > 3 indicates the fish are proportionally heavier for a given length (i.e. in better condition), whereas b < 3 indicates a fish that has proportionally less mass for its length (i.e. is in poorer condition) (Ricker 1975). Fulton's condition factor (i.e. b = 3) was used to allow comparisons with published literature (e.g. Dempson et al. 2008, Swanson et al. 2010). One of the main critiques of K is that it has an inherent length-bias (Rennie and Verdon 2008). Rennie and Verdon (2008) suggest that although K is useful for within-population, point-in-time comparisons, using K to consider trends over time, or compare among populations may result in significant bias due to size bias. Following Dempson et al. (2008), I calculated Fulton's condition factor for fish in the size range 450-599 mm to address this issue, and to remove the potential differences in condition between different size groups of Arctic char (Johnson 1980). Among-year variability in K was assessed with ANOVAs for each study site (Dempson et al. 2008). The assumption of slope homogeneity was not met, thus precluding the use

of ANCOVA for comparing length-weight relationships of Arctic char among years.

#### *3.2.5.4 Growth*

The growth curves of Arctic char based on length-at-age data were calculated with the von Bertalanffy growth model (VBGM; Eq. 9). This non-linear growth model has been demonstrated to best fit Arctic char populations in the Cumberland Sound region (Loewen et al. 2010).

**Eq. 9.** 
$$L_i = L_{\infty}[1 - e^{-k(i-t0)}]$$

In this equation,  $L_t$  is fork length, *i* is age (estimated from otoliths), k is the Brody growth coefficient,  $L_{\infty}$  is the mean asymptotic length, and  $t_0$  is the theoretical age at which length would be zero (Cerrato 1990, Isely and Grabowski 2007). Sample sizes were not large enough to allow growth curves to be calculated for all years individually, so data were divided into four-year periods: 1998-2001, 2002-2005, and 2006-2009. Data from 2011 were considered separately. The VBGM was fitted for each period within each study site using non-linear least squares (nls) in the nlstools package for R (R Core Development Team 2008; see Appendix B for coding). Likelihood ratio tests are considered to provide the best comparison of VBGM parameters (Cerrato 1990). Comparisons of fitted model parameters were thus compared between periods with likelihood ratio tests using the fishmethods package for R (R Core Development Team 2008; see Appendix C for coding).

#### **3.3 Results**

Biological data were gathered from 194 individual Arctic char from Kipisa and 200 from Isuituq, captured during the 2011 sampling season (Table 5). Sample sizes for

archived samples ranged from 46 to 214 individuals per year (Table 5). Length frequency distributions for each year are given in Fig. 16 (Kipisa) and Fig. 17 (Isuituq). There was year to year variability observed in the L<sub>F</sub> frequency distributions. ANOVAs on Ranks indicated significant differences in the median L<sub>F</sub> among years within both Kipisa (H = 18.951, df = 3, P < 0.001) and Isuituq (H = 39.296, df = 6, P < 0.001).



**Figure 16.** Length frequency distributions of Arctic char captured from Kipisa in (a) 2002, (b) 2003, (c) 2004, and (d) 2011.



**Figure 17.** Length frequency distributions of Arctic char captured from Isuituq in (a) 2002, (b) 2003, (c) 2004, (d) 2008, and (e) 2011.

#### 3.3.1 Stomach Contents

A total of 15 prey taxa were identified from 185 prey-containing stomachs collected from the two study sites (Kipisa: Table 6; Isuituq: Table 7). Owing to digestion, some stomach contents were unidentifiable. Important invertebrate prey were the crustaceans: Copepoda, *Mysis* spp., *Thysanoessa* spp., *Gammarus* spp., Lysianassoidea, and especially the hyperiid amphipod *Themisto* spp. Fish prey consisted entirely of sculpin (Cottidae) and capelin. Arctic char rarely fed on insects, polychaetes and decapods, and combined these taxa were found in only five stomachs. Non-animal debris, including small pebbles and terrestrial vegetation, was observed in a small proportion (4%) of the stomachs. For temporal comparisons, prey items were pooled into nine broad taxonomic groups: amphipods, capelin, copepods, euphausiids, insects, mysids, polychaetes, sculpin, and other.

In 2002, 2003, and 2004, amphipod crustaceans made up the highest proportion of the weight of Arctic char diets from both sites (Kipisa: Fig. 18; Isuituq: Fig. 19), and were the dominant prey item according to all three importance indices (Fig. 20). Capelin were absent from the stomachs in 2002, 2003, or 2004, but were the most important diet component (by % weight) in 2011, making the largest contribution to stomach contents for both Kipisa (79.8%; Fig. 18) and Isuituq (73.2%; Fig. 19). The capelin found in these stomachs were in varying stages of digestion, ranging from hard parts only (e.g. bones), to recently ingested, whole individuals. In 2011, capelin were the dominant prey item for Arctic char from Kipisa according to the IRI, whereas amphipods were still relatively important in the diets of Isuituq Arctic char (Fig. 20).

Arctic char diets were highly variable among years. For example, euphausiids made

up a high proportion of the diets of Kipisa fish in 2004 (30.4% by weight), but were absent from stomachs from Kipisa in 2002 (Fig. 18). Copepods were absent from 2004 and 2011 Isuituq stomachs, yet were the second most important prey item in 2003 (19.3% by weight), after amphipods (73.8%; Fig. 19).

Stomach contents of Arctic char varied between study systems (Fig. 20). Euphausiids were not found in any stomach from Isuituq, but were relatively important in those from Kipisa. Similarly, sculpin were a relatively important prey item for Kipisa Arctic char, but had low importance in all three indices for Arctic char from Isuituq, or were absent altogether. Arctic char from Kipisa seem to have a more varied diet, especially in 2003 and 2004, whereas Isuituq Arctic char relied mainly on amphipods.

The 2011 appearance of capelin was associated with a decrease in the importance of amphipods (Kipisa: Fig. 18; Isuituq: Fig. 19). *Themisto* spp. was the most important diet item for Kipisa Arctic char in the early 2000s, occurring in 100% of food-containing stomachs in 2003 and 2004, and yet this amphipod was found in only one stomach (4% occurrence) in 2011 (Table 6). In the stomachs of Isuituq Arctic char, *Themisto* spp. presence also decreased, ranging from 18.2% in 2004 to 71.4% in 2003 down to 2.3% in 2011 (Table 7). The abundance (by % weight) of amphipods in both study systems was markedly lower in 2011 than previous years. Amphipod % weight of stomachs in Kipisa ranged from a maximum of 92.2% in 2002 to a low of 1.3% in 2011, and in Isuituq ranged from 96.5% in 2004 to a low of 26.6% in 2011. However, amphipods remained important in terms of %O and %N for Arctic char from Isuituq (Table 7).

In stomachs from Kipisa, the %O of larval sculpin also appeared to decrease with capelin presence, decreasing from a maximum of 100% in 2004 to 16% in 2011.

However, the proportions of the prey taxa present in stomachs were highly variable among consecutive years for both Kipisa and Isuituq (Table 6 and Table 7, respectively). **Table 6.** Relative importance of diet items identified from the stomachs of anadromous Arctic char captured from Kipisa in 2002, 2003, 2004, and 2011. Diet indices represent percent occurrence (%O), percent representation by number (%N), and percent representation by weight (%W), calculated for each year. Immature and ambiguous individuals were not included in the analysis.

	2002	2002 2003			2004			2011				
Diet Item	%O	%N	%W	%O	%N	%W	%O	%N	%W	%O	%N	%W
Class Polychaeta				14.3	<0.1	0.1						
Class Insecta (Insects)												
Family Tipulidae (Crane-flies)	3.7	<0.1	<0.1									
Subphylum Crustacea												
Subclass Copepoda	59.3	5.2	0.7	57.1	1.2	3.9						
Family Mysidae												
<i>Mysis</i> spp.	11.1	0.5	<0.1	78.6	19.0	21.4	50.0	26.7	2.9	20.0	3.4	<0.1
Family Euphausiidae												
Thysanoessa spp.				35.7	0.1	0.7	94.4	15.5	30.4	12.0	80.3	18.7
Order Amphipoda												
Suborder Gammaridea												
Gammarus spp.	3.7	<0.1	0.1	7.1	0.7	<0.1						
Gammarellus homari	7.4	0.1	<0.1	7.1	<0.1	<0.1						
Superfamily Lysianassoidea				7.1	<0.1	<0.1				8.0	6.9	1.3
Suborder Hyperiidea												
Themisto libellula	66.7	92.4	91.9	92.9	80.7	57.6	100.0	45.1	49.1	4.0	0.2	0.1
Hyperia medusarum	7.4	0.1	0.1									
Suborder Corophiidae												
Ischyrocerus anguipes	7.4	0.6	0.1									
Order Decapoda ( <i>Eualus</i> spp.)	3.7	<0.1	0.1									
Unidentified crustacean remains	14.8	n/a	6.3	42.9	n/a	13.9	33.3	n/a	3.8			
Class Actinopterygii												
Family Cottidae (Sculpin)	22.2	1.1	0.6	85.7	0.4	1.2	100.0	12.7	13.7	16.0	0.3	0.1
Family Osmeridae												
Mallotus villosus (Capelin)										96.0	8.9	79.8
Unidentified fish remains				7.1	n/a	2.1						
Debris (non-animal)												

**Table 7.** Relative importance of diet items identified from the stomachs of anadromous Arctic char captured from Isuituq in 2002,2003, 2004, and 2011. Diet indices represent percent occurrence (O), percent representation by number (N), and percent representationby weight (W), calculated for each year. Arctic char with immature and ambiguous maturity status were not included in the analysis.

	2002			2003			2004			2011		
Diet Item	%0	%N	%W	%O	%N	%W	%O	%N	%W	%O	%N	%W
Class Insecta (Insects)												
Family Chironomidae (Non-biting Midges)				7.1	0.1	<0.1						
Unidentified Insect				7.1	<0.1	<0.1						
Subphylum Crustacea												
Subclass Copepoda	66.7	17.3	3.3	78.6	1.4	19.3						
Family Mysidae												
<i>Mysis</i> spp.	39.4	20.9	18.5	21.4	0.3	<0.1	18.2	0.1	<0.1	7.0	2.2	0.1
Order Amphipoda												
Suborder Gammaridea												
Gammarus spp.	18.2	1.6	0.5				45.5	9.2	8.1	16.3	14.9	4.9
Gammarellus homari												
Superfamily Lysianassoidea	51.5	49.2	58.6	14.3	0.1	<0.1	81.8	58.5	70.8	72.1	78.9	21.7
Suborder Hyperiidea												
Themisto libellula	30.3	9.0	8.3	71.4	98.1	73.8	18.2	31.9	17.7	2.3	0.1	<0.1
Unidentified crustacean remains	27.3	n/a	10.5	14.3	n/a	6.9	54.5	n/a	2.8	4.7	0.1	0.1
Class Actinopterygii												
Family Cottidae (Sculpin)	15.2	0.4	0.2	7.1	<0.1	<0.1						
Family Osmeridae												
Mallotus villosus (Capelin)										53.5	3.3	73.2
Debris (non-animal)	6.1	0.3	0.1	7.1	n/a	<0.1	9.1	0.1	0.6	9.3	0.5	<0.1



**Figure 18.** Relative importance (% wet weight) of prey items from the stomach contents of Arctic char captured from Kipisa in four different years. Data are from 84 preycontaining stomachs. Sample sizes (*n*) for each year were: 2002 n = 27; 2003 n = 14; 2004 n = 18; 2011 n = 25.



**Figure 19.** Relative importance (% wet weight) of prey items from the stomach contents of Arctic char captured from Isuituq in four different years. Data are from 101 preycontaining stomachs. Sample sizes (*n*) for each year were: 2002 n = 33; 2003 n = 14; 2004 n = 11; 2011 n = 43.



**Figure 20.** Relative importance (%IRI) of major prey categories identified from stomach content data of Arctic char from Kipisa (K) and Isuituq (I) captured in 2002, 2003, 2004, and 2011. Data are from 84 (K) and 101 (I) prey-containing stomachs. Sample sizes (*n*) each year were: 2002  $n_k = 27$ ,  $n_i = 33$ ; 2003  $n_k = 14$ ,  $n_i = 14$ ; 2004  $n_k = 18$ ,  $n_i = 11$   $n_k = 25$ ,  $n_i = 43$ .

#### 3.3.2 Stable Isotopes

The  $\delta^{15}$ N of Arctic char muscle varied significantly among the years sampled, for both Kipisa ( $F_{4,104}$ =20.08, P < 0.001) and Isuituq ( $F_{5,174}$  = 23.52, P < 0.001) (Fig. 21a). The  $\delta^{15}$ N adjusted means ranged from 13.65‰ in 2004 (Kipisa) to 15.04‰ in 2011 (Isuituq). There were no consistent trends in  $\delta^{15}$ N apparent over time. Tukey's HSD test showed a significant difference between 2004 and 2011 for both study sites (P < 0.05), but not between 2003 and 2011 for Arctic char from Kipisa, and not between 2002 and 2011 for Arctic char from Isuituq. The values of  $\delta^{13}$ C ranged from -20.50‰ in 2002 (Kipisa) to -18.80‰ in 2011 (Isuituq), and were significantly different among years for both systems (Kipisa:  $F_{3,105} = 157.998$ , P < 0.001; Isuituq:  $F_{4,178} = 56.2$ , P < 0.001) (Fig. 21b). As apparent in Fig. 21b, there was a general trend towards lower  $\delta^{13}$ C over time within both study systems, with significantly higher  $\delta^{13}$ C in 2011 in comparison to all previous sampling years (P < 0.05). The trend was inconsistent, however, as Isuituq Arctic char captured in 2008 had significantly lower  $\delta^{13}$ C compared to those captured in 2004.

Arctic char from Isuituq had significantly higher  $\delta^{15}$ N compared to fish from Kipisa in each year except 2003 (P < 0.05), with an average difference of 0.55‰. Isuituq Arctic char also had significantly higher  $\delta^{13}$ C compared to those from Kipisa (P < 0.05), except for fish captured in 2011, when  $\delta^{13}$ C for both sites converged at -18.8‰.

#### 3.3.3 Condition and Growth

Student's *t*-tests indicated no significant difference in Fulton's condition factor (K) between males and females; therefore, sexes were pooled for all analyses. From preliminary data analysis it was evident that K calculated using somatic weight (i.e. gonad weight subtracted from whole body weight) were not significantly different from K calculated using total body weight. Because gonad weights were not available for all sample years, condition factor was calculated from fresh whole body weight (g) to allow for an extended timeline and larger sample sizes.

Condition factor (K) varied significantly among years for both study sites (Kipisa:  $F_{5,400} = 12.684$ , P < 0.001; Isuituq:  $F_{6,522} = 15.136$ , P < 0.001) (Fig. 22). The K (mean ± SEM) of fish from Kipisa ranged from  $1.18 \pm 0.03$  in 2002 to  $1.36 \pm 0.02$  in 2003 and from  $1.12 \pm 0.02$  in 2011 to  $1.28 \pm 0.01$  in 2006 for fish from Isuituq. The condition of Arctic char from Isuituq appeared to increase yearly until 2005, and then began to decrease. *Post hoc* multiple comparison tests showed that fish condition in Isuituq was significantly lower in 2011 than all previous years (P < 0.05). Differences in condition were also observed between study sites: the K of Kipisa fish was significantly higher than Isuituq fish for 2003, 2004, and 2011(P < 0.05), but not for 2002.

The estimated parameters for the von Bertalanffy growth model (VBGM) fitted to length-at-age data derived for each period are given in Table 8, and fitted curves in Fig. 23 and Fig. 24. The likelihood ratio test revealed no significant differences were evident in the VBGM parameters between the 1998-2001 and 2002-2005 periods for the Kipisa Arctic char population, whereas  $L_{\infty}$  and K were larger and smaller, respectively, in 2011 compared to both previous periods (P < 0.05). It must be noted that the sample size for 2011 was relatively small and there were fewer large, older (> 15 years) fish represented in the 2011 sample compared to the other periods. The VBGM parameters for the Isuituq populations were not significantly different between the 2006-2009 and 2011 periods, but there was significantly lower t<sub>0</sub> between both 2002-2005 and 2006-2009, and 2002-2005 and 2011. Explicit results of likelihood ratio tests ( $\chi^2$ , df, and *P*-value) between periods are given in Appendix D.



**Figure 21.** Nitrogen (a) and carbon (b) stable isotope ratios (mean  $\pm$  SE) of Arctic char muscle tissue from two open lake systems – Kipisa and Isuituq – in 2002-2004, 2008 (Isuituq only), and 2011 (see Table 1).



**Figure 22.** Mean ( $\pm$  SE) Fulton's condition factor (K) for Arctic char with a fork length of 450-599 mm from 1998 to 2011 for two study sites (Kipisa and Isuituq) within the Cumberland Sound region of Nunavut.

**Table 8.** Estimated parameters and associated standard errors (SE) of the von Bertalanffy growth model (VBGM) fitted to Arctic char length-at-age data from two study sites (Kipisa and Isuituq) and four periods (1998-2001, 2002-2005, 2006-2009, 2010-2011). Note that data were not available for all years for both systems.

System	Period	n	L∞	SE	К	SE	t <sub>0</sub>	SE
Kipisa	1998-2001	349	691	19.1	0.201	0.032	2.479	0.733
	2002-2005 2006-2009	344	702	29.3	0.163	0.033	1.454	0.899
	2010-2011	194	937	141.3	0.072	0.020	0.218	0.668
Isuituq	1998-2001							
	2002-2005	645	691	24.6	0.142	0.023	0.112	0.858
	2006-2009	370	706	12.7	0.188	0.015	2.176	0.255
	2010-2011	200	690	20.4	0.168	0.017	2.174	0.316





**Figure 23.** Length-at-age data fitted with the von Bertalanffy growth model from the Kipisa Arctic char population during three periods: a) 1998-2001, b) 2002-2005, and c) 2011. Dashed lines indicate the 95% upper and lower confidence intervals.





**Figure 24.** Length-at-age data fitted with the von Bertalanffy growth model from the Isuituq Arctic char population during three periods: a) 2002-2005, b) 2006-2009, and c) 2011. Dashed lines indicate the 95% upper and lower confidence intervals.

#### **3.4 Discussion**

This study provides the first evidence of capelin in the diet of Arctic char in Cumberland Sound, revealing a diet shift that has occurred in less than a decade. My data suggest that this shift occurred between 2005 and 2011. Similar diet shifts have been observed in other Arctic char populations. Dempson et al. (2002) reported a decline in the presence of capelin in the diets of Arctic char from Labrador, Canada during the late 1990s. In Norway, Rikardsen et al. (2007) found that there was a marked change in the marine diet of Arctic char between 1992-1993 and 2000-2004, when Arctic char began to rely on a piscivorous diet of herring as opposed to their previous diet of zooplankton. This finding led Rikardsen et al. (2007) to suggest that in general Arctic char diets reflect prey availability in the marine environment, due to an opportunistic foraging strategy. However, Rikardsen et al. (2007) also proposed that Arctic char feed preferentially on herring, despite the availability of other prey. The high importance of capelin in the diet of Cumberland Sound Arctic char in 2011 and the low diet diversity in 2011 leads to questions of whether Arctic char are merely taking advantage of a highly available prey item or whether capelin is a preferred prey species. Additionally, there is the possibility that the arrival of capelin in this ecosystem has led to a decrease in the historical prey of Arctic char.

Capelin are planktivorous fish and although their diet in Cumberland Sound has not been investigated, recent research from West Greenland revealed that euphausiids, amphipods, and copepods were the most important prey (by weight) for capelin (Hedeholm et al. 2012). Similarly, Petursdottir et al. (2012) reported copepods and the amphipod *Themisto* as important capelin diet items in the Iceland Sea. It is significant that these invertebrates were found to be important for Arctic char within Cumberland Sound in previous years, as found in this study. One of the most prevalent species in the diets of Arctic char during the pre-capelin years in this study, *Themisto* spp., was also one of the most common prey species for capelin (Hedeholm et al. 2012). Furthermore, a link between capelin and *Themisto* populations has been documented in the Barents Sea by Dalpadado et al. (2001). The authors attributed an increase in *Themisto* abundance during the mid-1980s to reduced predation by capelin, which experienced a population decline during the same period. Perhaps a combined effect of increased capelin abundance and decreased availability of invertebrate prey in Cumberland Sound has contributed to the diet shift observed in Cumberland Sound Arctic char.

The high variability in prey composition observed from year to year is indicative of an opportunistic feeding strategy of Arctic char, as has been observed by other researchers (Rikardsen and Amundsen 2005). A flexible, opportunistic foraging strategy is likely optimal for Arctic char to achieve the high rates of weight and lipid gain exhibited by anadromous individuals during the marine feeding period, as individuals would be able to opportunistically exploit high density prey patches (Rikardsen and Amundsen 2005). This was suggested by Rikardsen and Amundsen (2005), who observed Arctic char feeding up to 5000 m offshore, and attributed this behaviour to the Arctic char taking advantage of high densities of herring. High density prey aggregations are common in polar marine systems, observed in amphipods (Dalpadado et al. 2001), euphausiids (Zhou et al. 2005), and copepods (Trudnowska et al. 2012). Similarly, capelin exhibit aggregating behaviour that is especially evident during pre-spawn, spawning, and post-spawning (Davoren et al. 2003). Personal observations of capelin spawning along the intertidal zone in Cumberland Sound fiords in June and July suggests that capelin aggregations may be a particularly important food source for Arctic char at this time of year, as this is also when the Arctic char have just begun their intensive marine feeding season. The Arctic char of Cumberland Sound are thought to begin entering salt water even prior to the melting of river ice, starting as early as mid-May (Moore 1975a), similar to other Arctic regions (Johnson 1980).

Despite having a wide, or generalist, trophic niche at the population level, as was observed here in pre-capelin years, it has been suggested that individual Arctic char have a more specialized trophic niche (Amundsen 1995). From work with seabirds (Brünnich's guillemot *Uria lomvia*), Woo et al. (2009) propose that generalist and specialist foraging

strategies are dependent on resource availability. A generalist strategy is hypothesized to be a fitness advantage in environments when resources are variable in abundance or either spatially or temporally, whereas specialists would benefit when resources were more homogeneous and predictable (Paszkowski 1984, Woo et al. 2009). As Arctic char are known to be able to exhibit trophic specialization even within short periods (Michaud et al. 2008), and as stomach content data from 2011 seems to suggest, it is possible that some individuals have begun to specialize on the presence of capelin in the Cumberland Sound region.

Variation in the length frequencies of the population samples likely did not contribute to the observed variability in diet composition among pre-capelin years, as Dempson et al. (2002) noted no effect of predator size for large (> 300 mm  $L_F$ ) fish in years when capelin were absent. Similarly, there was not much difference in diet composition among size classes of Arctic char larger than 350 mm, as reported by Moore and Moore (1974).

The first documentation of capelin in Cumberland Sound was inadvertently provided by Dennard et al. (2008), who investigated the potential significance of capelin to Greenland halibut (*Reinhardtius hippoglossoides*). The authors reported the stable isotope ratios for capelin in Cumberland Sound as -19.25‰ for  $\delta^{13}$ C and 12.92‰ for  $\delta^{15}$ N (Dennard et al. 2008). Similar results were reported by Marcoux et al. (2012) for capelin captured from Cumberland Sound in 2007-2009 (-19.52‰ for  $\delta^{13}$ C and 13.83‰ for  $\delta^{15}$ N). Given that trophic fractionation of  $\delta^{15}$ N is approximately 3-4‰ (Vander Zanden and Rasmussen 1999), Arctic char that feed primarily on capelin with  $\delta^{15}$ N of 12.9-13.8‰ would be expected to have an isotopic signature ranging from 15.9 to 17.8‰. The

 $\delta^{15}$ N observed in 2011 was somewhat lower (14.4-15.0‰), which likely reflects the inclusion of invertebrate prey, as were found in the stomach contents.

The lack of significant change in  $\delta^{15}$ N over time must be considered with regard to the  $\delta^{15}$ N of potential prey. Hobson and Welch (1992) assigned capelin to a trophic position of 3.0 relative to copepods, based on a study of the Arctic marine food web in Barrow Strait-Lancaster Sound. The results of the Hobson and Welch (1992) study place most of the other potential diet items of Cumberland Sound Arctic char at lower trophic positions than capelin, such as mysids ( $\delta^{15}$ N: 10.3 ± 0.3‰) and the amphipod *Parathemisto libellula* ( $\delta^{13}$ C: -20.3 ± 0.4‰ and  $\delta^{15}$ N: 11.7 ± 0.7‰). These two species have both been reported in Cumberland Sound Arctic char diets (Moore and Moore 1974), and were placed at trophic positions of 2.3 and 2.7, respectively (Hobson and Welch 1992). In contrast, Petursdottir et al. (2012) reported that capelin and *Themisto* from the Iceland Sea were virtually indistinguishable in trophic level. In the absence of prey information, I can only speculate that there is minimal difference in stable isotope ratios between the past and present prey items for Arctic char in Cumberland Sound.

Baseline levels of  $\delta^{13}$ C and  $\delta^{15}$ N can be variable between and within ecosystems, introducing potential bias when interpreting trophic positions and comparing among systems (Vander Zanden et al. 1997, Post et al. 2002). Marcoux et al. (2012) assessed the stable isotopes of beluga (*Delphinapterus leucas*) in Cumberland Sound over the past two decades, and observed declines of both  $\delta^{13}$ C and  $\delta^{15}$ N, which led the authors to suggest either a trophic change or a shift in baseline stable isotope ratios. The  $\delta^{13}$ C decrease found by Marcoux et al. (2012) is opposite to the observations in the present study. Throughout the same period (2004 to 2011), the anadromous and resident components of

two Arctic char populations in the Cumberland Sound region experienced similar changes in  $\delta^{13}$ C (i.e.  $\delta^{13}$ C increased), by 0.55‰ (see Chapter 1). Because resident and anadromous Arctic char generally do not feed on the same diet items, this change might be accounted for by a shift in the  $\delta^{13}$ C baseline. However, the convergence of  $\delta^{13}$ C for both systems in 2011 suggests that these Arctic char are feeding on prey that have acquired energy from the same carbon source.

The condition of Arctic char from Kipisa and Isuituq fall within the range (1.0 to 1.3) previously reported for Arctic char in the Canadian Arctic (DFO 2010, Swanson et al. 2010). Fish from Kipisa were at the high end of that range in 2003 and 2004, which suggests a marine feeding season in these years. Interestingly, the diet composition in these years was most variable, with relatively high importance of sculpin and euphausiids. The variability among years has also been reported previously (DFO 2010), and although fish were in somewhat poorer condition in 2011, not enough data exist to draw conclusions that it is significant or related to foraging on capelin.

Growth models indicate change in the growth of these Arctic char populations between late 1990s to early 2000s and more recent years (2006 and later), although the parameter changes were not consistent between the two study sites. The finding of increased size-at-age (Isuituq) following the availability of capelin in Cumberland Sound is in accordance with Dempson et al. (2008), who reported a decreased size-at-age for Arctic char from Labrador during a period in which capelin were essentially absent from the region. However, unlike Dempson et al. (2008) the present study only provides a relatively short timeline for observing growth effects. In addition, the sample sizes for 2011 are relatively small compared to the other periods. Cerrato (1990) claimed that

sample sizes smaller than n = 300 may produce inaccurate results when applying the von Bertalanffy growth model.

Although not conclusive, my results suggest that foraging on capelin may be positively affecting the growth of Arctic char in Cumberland Sound. In general, fish growth and survival are dependent on food quality and quantity (Daly et al. 2010), both of which may be provided by high density aggregations of capelin. Capelin represent a high-lipid prey item (Lawson et al. 1998) that may provide benefits for Arctic char in terms of growth and condition (Michaud et al. 2010). As discussed above, capelin are perhaps most available during the period in which Arctic char have the greatest need for a high-lipid food source: upon entering the marine environment after the long winter months of fasting. Another factor that may have affected growth is change in water temperature, which is implicated by the changes in capelin distribution (Rose 2005). Michaud et al. (2010) suggest that water temperature and capelin availability are linked. Higher sea water temperatures have been linked with higher growth in chum salmon (Oncorhynchus keta) (Seo et al. 2011), and may provide Arctic char a longer period within their optimal growth temperature (Reist et al. 2006). Therefore, any changes observed in Arctic char growth may reflect a combination of these two factors.

Changes in the growth and condition of Arctic char in this region also have significant economic and cultural implications. In the Canadian Arctic, Arctic char are harvested for subsistence purposes by Inuit (DFO 2010), forming a significant part of their diet and traditional lifestyle. In addition, some Arctic communities, such as the hamlets of Pangnirtung, Cambridge Bay, and Rankin Inlet, are economically invested in commercial Arctic char fisheries, equipped with fish processing infrastructure (Rompkey

and Patterson 2010). Considering the increasing economic reliance on these fisheries, steps need to be taken to ensure management practices promote the health of Arctic char populations. These should include prioritizing research that investigates factors that have potential impacts on Arctic char populations, including trophic ecology (Brown et al. 2010). Ecosystem-based management is fast becoming the goal for fisheries worldwide, of which modeling of food webs is an important component (Gaichas et al. 2010). Providing basic ecological information on Arctic char trophic strategies, such as those provided in this study, will contribute to assessing other changes in Arctic systems, particularly the effects climate change and shifts in species distributions. This information will aid fisheries managers in making informed decisions.

# Chapter 4 The influences of lipids and lipid extraction on carbon, nitrogen, and sulphur stable isotope signatures in a partially migratory Arctic fish species, the Arctic char

# Abstract

In response to inconsistencies in the literature and to confusion as to whether or not to extract lipids prior to stable isotope analysis, this research was undertaken to assess effects of lipids and lipid extraction on the stable isotope ratios of carbon, nitrogen, and sulphur of a partially migratory fish species, the Arctic char. Stable isotope analysis of  $\delta^{13}$ C,  $\delta^{15}$ N and  $\delta^{34}$ S was conducted on muscle tissue samples Arctic char in bulk (lipidcontaining) and lipid extracted (LE) forms. To assess the differences between high lipid and low lipid samples within the same study species, two Arctic char ecotypes: resident (i.e. non-migratory) and anadromous (i.e. migratory), were studied. Chemical lipid extraction and four lipid normalization models were tested. Lipid content of the tissue was found to have a statistically significant affect on  $\delta^{13}$ C, and the extraction of lipids significantly affected  $\delta^{15}$ N and  $\delta^{34}$ S. Chemical extraction effectively reduced C:N ratios – a proxy for lipid content – and was determined to be more reliable for alleviating lipid bias on  $\delta^{13}$ C than arithmetic normalization. It is recommended that  $\delta^{15}$ N and  $\delta^{34}$ S should be analysed from bulk tissue, whereas  $\delta^{13}$ C should be analysed from lipid-extracted tissue. The use of normalization models to correct  $\delta^{13}$ C is not recommended without species- and ecotype-specific validation.

### 4.1 Introduction

Stable isotopes are widely used for ecological purposes. In fishes, the stable isotopes of carbon, nitrogen, and, to a lesser extent, sulphur, are used to identify feeding niches (Adams et al. 2003), define habitat use (Doucett et al. 1999), and follow energy pathways

through ecosystems (i.e. food webs; Vander Zanden and Rasmussen 1996). Although the methods and techniques for accomplishing these analyses are relatively standardized, there remains uncertainty over two questions critical to the correct interpretation of these data: (1) whether or not lipids should be accounted for when performing stable isotope analysis with a particular sample set, and (2) if lipid correction is to occur, what method is most appropriate (Post et al. 2007). Although others have attempted to assess these questions (McConnaughey and McRoy 1979, Sotiropolous et al. 2004, Kiljunen et al. 2006), and it seems that consensus has been reached that lipid correction is necessary in many cases (Mintenbeck et al. 2008), these requirements have yet to be validated for individual species. Assessment of the requirements and procedures for lipid correction is necessary in Assessment of the requirements and procedures for lipid correction is necessary.

In fish, lipids – in particular, the fatty acid components of triacylglycerols – represent the main source of metabolic energy for growth and development (Sargent et al. 1999), as well as for reproduction and migration (Tocher 2003). Fish obtain the majority of these requisite lipids through their diets; however, lipid modification and biosynthesis are sometimes necessary for freshwater fish to fulfil their metabolic requirements (Tocher 2003). Despite the importance of lipids in fish diets and overall biology, they can become problematic when using stable isotopes as a tool for ecological research if not addressed appropriately (Post et al. 2007). Lipids have lower  $\delta^{13}$ C compared to other tissue constituents, such as proteins and carbohydrates, due to processes during lipid biosynthesis (DeNiro and Epstein 1977, Mintenbeck et al. 2008). White muscle is the most widely used tissue for stable isotope analysis in fish ecological research, which

necessarily contains proteins as well as lipids to varying degrees among species (Tocher 2003). Because lipids have a disproportionate amount of the heavier carbon isotope ( $^{13}$ C) compared to other tissue constituents, it would thus be erroneous to compare  $\delta^{13}$ C among samples with significantly different lipid quantities (Post et al. 2007).

In order to counter the effect of lipids on  $\delta^{13}$ C, two approaches have been proposed. The first solution is to remove the lipid molecules; this is generally done with a chloroform-methanol reagent, using procedures such as Bligh and Dyer (1959) or Folch et al. (1957). Although this seems to be a relatively straight-forward solution, lipid extraction introduces a new set of problems. Not only is lipid extraction time-consuming, but there is evidence that chemical lipid removal may have an effect on the measured  $\delta^{15}$ N of the tissue (Post et al. 2007). In contrast to  $\delta^{13}$ C,  $\delta^{15}$ N is not affected by lipid composition; however, several authors have suggested that the procedures for lipid extraction alter the nitrogen isotope signature: Post et al. (2007) found that lipid extraction led to nitrogen isotope fractionation of approximately 0.25%, and even larger increases were reported by Mintenbeck et al. (2008) and Sotiropolous et al. (2004), who found changes of 1.65‰ and 2.8‰, respectively. Accordingly, samples for isotope analysis may need to be run in duplicate: in untreated (i.e. bulk), and in lipid extracted forms, thereby increasing the costs (approximately \$10 to \$25 per sample depending on the isotope and the degree of sample preparation completed) and time required for analysis. Based on a study including aquatic animals, terrestrial animals, and plants, Post et al. (2007) have suggested using the C:N ratios of bulk samples to determine whether lipid extraction is necessary, because C:N have been used as predictors of lipid levels. They recommend that C:N ratios that are high (> 4.0) or variable among samples indicate
high or variable levels of lipids among samples, signaling the need to treat for lipids.

Given that lipid extraction has a possible effect on the nitrogen isotope, it follows that the chemical extraction process may also affect  $\delta^{34}$ S. To date, the sulphur stable isotope ratio is used mainly for trophic source differentiation in food web studies (Peterson and Howarth 1987), determining migratory patterns (Hesslein et al. 1993), and tracking habitat use (Fry and Chumcal 2011). The benefits of using  $\delta^{34}$ S include lack of fractionation across trophic levels and wide differences in primary producers from different environments (Peterson and Howarth 1987), and for fish in particular are considered to being minimally affected by factors such as temperature, body size, and age (Barnes and Jennings 2007). Despite the current uses and benefits of conducting sulphur stable analysis in aquatic systems and for fish in particular, and the importance of accounting for lipids when conducting  $\delta^{13}$ C and  $\delta^{15}$ N analyses, there have been no published studies on the effects of lipid extraction on  $\delta^{34}$ S.

The alternative method proposed for dealing with lipid bias on  $\delta^{13}$ C is the application of *post hoc* normalization equations, which have the benefit of reduced cost and time expenditures. There have been several attempts to create correction factors for  $\delta^{13}$ C in lipid-containing samples (McConnaughey and McRoy 1979, Kiljunen et al. 2006, Post et al. 2007), which would eliminate the need for duplicate analysis (i.e. bulk and lipid extracted) of each sample. These equations estimate lipid content based on its relationship with C:N (Mintenbeck et al. 2008). The difficulty, however, is that these corrections are often based on groups of organisms, and have not been validated for species, despite apparent inter-population variability in the relationship between C:N and lipid content (Fagan et al. 2011). Does this indicate that each population must then be

treated separately, with an individually-derived normalization model? With intrapopulation variability present within some species – for instance, the migratory ecotypes of Arctic char (see Chapter 1 Section 1.3) – I wondered whether it was appropriate to apply a single normalization model, or if chemical lipid extraction was required for accurate results.

This research addressed two main objectives: (1) to determine how lipid extraction affects the stable isotope signatures of Arctic char, and (2) to assess if a lipid-correction model can be applied to this species. The research was conducted on muscle tissue from Arctic char within the geographical region of Cumberland Sound, where multiple populations and at least two ecotypes exist (see Chapter 1 Section 1.3). The effects of lipid extraction on isotope composition was investigated for four Arctic char populations, two of which consist of anadromous (sea-run) and resident (freshwater only) ecotypes. Arctic char provide an interesting comparison of low-lipid and high-lipid individuals due to the coexistence of two ecotypes within a single population (i.e. there is thought to be gene flow between the ecotypes [Loewen et al. 2010]) with distinctly different life history strategies (Loewen et al. 2009). The anadromous individuals undertake annual migrations to salt water for an intensive feeding period in the summer months, returning to fresh water for spawning and/or overwintering in the fall. Resident fish, on the other hand, remain in fresh water year-round. The freshwater lakes in this region have few vertebrate species (DFO 2010, 2005), and the small maximum size of residents is likely the result of limited trophic resources in the freshwater environment (Gross et al. 1988). Given that no published reports exist for the effects of lipid extraction on  $\delta^{34}S$ , a subsample of two of the populations was selected for sulphur analysis as well as carbon and nitrogen.

#### 4.2 Materials and Methods

#### 4.2.1 Animals

The samples used in this study were obtained from archived Arctic char muscle tissue. Sampling was conducted from July to September in 2002, 2003, 2004, and 2008 in four lake systems in the Cumberland Sound region of Nunavut, Canada: Kipisa (PG004), Qasigiyat (PG015), Isuituq (PG080), and Iqalugaarjuit (PG082) (Fig. 1 and Fig. 12). Fish were collected with gillnets in the lakes or at the estuarine mouth of the connecting rivers or streams. Details of the study sites and fish sampling procedures can be found in Chapter 2 and Chapter 3 of this thesis (see Section 2.2.2 and Section 3.2).

Ecotypes (anadromous and resident) were previously assigned to the archived samples on the basis of otolith strontium profiles, morphological characteristics, and life history for Qasigiyat and Iqalugaarjuit (Loewen 2008), and on location of capture for Kipisa and Isuituq (DFO 2005, 2010). The trophic niche of Arctic char is known to shift with ontogenetic stage (Forseth et al. 1994, Eloranta et al. 2010); therefore, to control for ontogenetic differences among individuals, only clearly distinguishable adult fish (i.e. those with a maturity status of mature, ripe, resting, or spent) were included in the analyses.

## 4.2.2 Laboratory Analysis

An  $\approx$  3 g subsample of muscle tissue was prepared for analysis by freeze-drying for a minimum of 48 hours and then grinding to a fine powder with a mortar and pestle (McMeans et al. 2010). A 0.5 ml subsample of the dried and ground tissue was portioned into a 5 ml cryovial and lipid extracted using a modified Bligh and Dyer (1959) method, as follows. Approximately 4 ml of a 2:1 chloroform methanol (CHCl<sub>3</sub>:MeOH) solution

was added to the tissue. The sample was vortexed for 15 seconds and placed in a 30°C water bath for 24 hours. After centrifuging for 6 minutes, the supernatant was decanted and discarded, and another 4 ml of 2:1 CHCl<sub>3</sub>:MeOH was added to the sample. The sample was again vortexed for 15 seconds, centrifuged for 6 minutes, and the supernatant removed. The remaining solids were left to dry for 48 hours in a fume hood.

For each treatment (bulk and lipid extracted), I measured 0.40-0.60 mg into preweighed tin capsules for  $\delta^{13}$ C and  $\delta^{15}$ N analysis, and 2.0-2.4 mg for sulphur analysis. Analyses of the carbon and nitrogen isotopes were conducted simultaneously with a Delta V Advantage continuous-flow isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) coupled to a 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA) at the Great Lakes Institute of Environmental Research (University of Windsor, Ontario). Every thirteenth sample was run in triplicate, and four internal laboratory standards and one National Institute of Standards and Technology (NIST) reference standards were run after every 15 samples for quantification. During this analysis, analytical precision for  $\delta^{15}$ N, based on the standard deviation of a lab standard (fish muscle) and NIST standard 8414 (bovine liver), was 0.15 and 0.14‰, respectively, and for  $\delta^{13}$ C was 0.14 and 0.10‰, respectively.

Sulphur analysis was conducted with an Isochrom Continuous Flow Stable Isotope Ratio Mass Spectrometer (GVInstruments/Micromass, UK) coupled to a Costech Elemental Analyzer (CNSO 4010, UK). Two NIST organic sulphur materials, Bovine Liver and Mussel, were run for corrections. Every tenth sample was run in duplicate, with a standard error of  $\pm 0.08\%$ .

In accordance with standard practices, stable isotope ratios are given as the

difference in the isotopic ratio ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{34}$ S) between the sample and reference standards (Peterson and Fry 1987). The ratios are expressed as parts per thousand (‰) following the formula:

$$\delta^{13}C, \ \delta^{15}N, or \ \delta^{34}S = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000$$
Eq. 1.

where R = the ratio of carbon (<sup>13</sup>C: <sup>12</sup>C), nitrogen (<sup>15</sup>N: <sup>14</sup>N), or sulphur (<sup>34</sup>S:<sup>32</sup>S) isotopes of the sample. By convention, standards are set at 0‰. The change ( $\Delta$ ) in each variable following lipid extraction was calculated as:

# Eq. 2. $\Delta \mathbf{X} = \mathbf{X}_{LE} - \mathbf{X}_{E}$

where  $X = \delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^{34}S$ , or C:N; LE = lipid extracted (treated) ; and B = bulk (untreated).

## 4.2.3 Model Evaluation

Three lipid normalization models were evaluated for their efficacy in adjusting  $\delta^{13}$ C to account for lipid bias for Arctic char. Resident and anadromous fish were assessed separately due to apparent differences in life history, habitat, and trophic niche (Loewen et al. 2009, 2010; Chapter 2).

The model proposed by McConnaughey and McRoy (1979) is given by Eq. 10 and 11.

Eq. 11. 
$$L = \left[ \frac{93}{[1 + (0.296 \times \text{C}: \text{N} - 0.775)]^{-1}} \right]$$

$$\delta^{13}C' = \left. \delta^{13}C + D \times \left[ l + 3.90 \right] / \left( 1 + \frac{287}{L} \right) \right]$$
  
Eq. 12.

where lipid content (*L*) is determined using the carbon to nitrogen ratio (C:N), and where *D* and *I* are constants used to determine the lipid-normalized  $\delta^{13}$ C ( $\delta^{13}$ C'). *D* refers to the  $\delta^{13}$ C difference between samples containing pure protein and pure lipid. McConnaughey and McRoy (1979) assigned constant values of 6% (*D*) and -0.207 (*I*). C:N and  $\delta^{13}$ C are measured values from the untreated (bulk) sample.

Kiljunen et al. (2006) revised the model given by Eq. 11 and 12, where the basic assumptions remain the same, but the constants are modified as D = 7.018 and I = 0.048.

A linear relationship between C:N and  $\delta^{13}$ C is the basis of the normalization equation proposed by Post et al. (2007) (Eq. 13).

Eq. 13. 
$$\delta^{13}C' = \delta^{13}C - 3.32 + 0.99 \times C:N$$

where C:N is of untreated (bulk) tissue and is assumed to be directly proportional to lipid content.

Sweeting et al. (2006) presented a mass-balance alternative to the previous equations, where bulk samples are assumed to consist of lipid and protein only (i.e. the carbohydrate component is negligible), and the difference in  $\delta^{13}$ C between pure lipid and pure protein was estimated at 7‰ (Eq. 14). For this model, a subsample of lipid-extracted samples are required in order to estimate the C:N of "pure" protein.

Eq. 14. 
$$\delta^{13}C_{\text{protein}} = \frac{(\delta^{13}C_{\text{bulk}} \times C: N_{\text{bulk}}) + (7 \times (C: N_{\text{bulk}} - C: N_{\text{protein}}))}{C: N_{\text{bulk}}}$$

#### 4.2.4 Statistical Analysis

Statistical analyses were conducted using SigmaStat v.3.5 (Systat Software Inc. 2006). Student`s *t*-tests, paired *t*-tests, and analysis of variance (ANOVA) tests followed

by Tukey's HSD test were used when appropriate to determine statistically significant differences. In addition, the coefficient of variation (CV; i.e. the percentage of variation in the mean) was used to assess the effect of lipid extraction on the variation of each variable (Sokal and Rohlf 1995, Mintenbeck et al. 2008). This measure of dispersion is dimensionless (Zar 1999), thus can be compared among variables. Standard error ( $\pm$  SEM) was reported whenever means were given. The parametric assumptions of normality and homogeneity of variances were tested with the Kolmogorov-Smirnov test and examination of residuals (Zar 1999). When these basic assumptions were not met, data were log transformed and reassessed. The non-parametric equivalent test was used when transformation failed to normalize the data (e.g. Wilcoxon signed rank, Kruskal-Wallis ANOVA on Ranks, Mann-Whitney *U*; Zar 1999). Significance was set at 0.05.

Differences among ecotype, year, and study site were tested statistically. Ecotypes were compared for the lake systems from which both anadromous and resident fish were sampled: Qasigiyat and Iqalugaarjuit. Year-specific analyses were assessed from the study sites with multi-year data: Kipisa and Isuituq. Study site differences were compared among samples collected in 2004, because all sites were sampled in this year.

The performance of published lipid-correction models when applied to these data was evaluated by simple linear regression, including calculation of the *F*-statistic for slope = 1 and intercept = 0 to detect bias (Mayer and Butler 1993). Paired *t*-tests were used to test for similarity of paired observed and model-predicted  $\delta^{13}$ C. Model performance was also compared using modelling efficiency (Eq. 15).

$$EF = 1 - \sum \frac{(y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y}_i)^2}$$

Eq. 15.

Modelling efficiency (*EF*) was calculated from the  $y_{i}$ , the observed  $\delta^{13}$ C (i.e. from lipid extracted samples), and  $\hat{y}_i$ , the predicted  $\delta^{13}$ C ( $\delta^{13}$ C'). This equation evaluates the goodness of fit of the model by measuring the proportion of the variation explained by the 1:1 line (i.e.  $y_i = \hat{y}_i$ ), where a value of 1 indicates a perfect fit and values approaching or below zero indicate a poor fit (Mayer and Butler 1993).

#### 4.3 Results

The samples analysed for each ecotype, location, and collection year are given in Table 9 for the carbon and nitrogen stable isotopes and Table 10 for the sulphur stable isotope. The  $\delta^{15}$ N,  $\delta^{13}$ C, and  $\delta^{34}$ S of Arctic char white muscle tissue were significantly affected by lipid extraction, although the extent of this effect for  $\delta^{13}$ C depended on bulk tissue C:N. Ecotypes differed in the magnitude and variation in C:N, providing an informative within-species comparison.

The mean ( $\pm$  SEM) C:N of untreated tissue ranged from 3.60  $\pm$  0.04 (Qasigiyat residents) to 4.98  $\pm$  0.31 (Kipisa anadromous) (Table 9). Anadromous fish had higher C:N than residents, with significant differences in mean C:N among ecotypes (*F* = 14.465, df = 3, *P* < 0.001). Differences in C:N were significant between but not within ecotypes, regardless of study site (*P* < 0.05). Anadromous C:N<sub>bulk</sub> was somewhat different among study sites (H = 7.398, df = 3, *P* = 0.060), but did not differ among years (H = 4.617, df = 3, *P* = 0.202).

Differences in the muscle tissue of resident and anadromous ecotypes were obvious during tissue sample preparation for stable isotope analysis. Samples that were more difficult to homogenize (i.e. seemed oily) were consistently among the samples with higher bulk C:N. Samples that were easy to crush were often pale in colour and were among the samples with lower C:N. Differences between ecotypes were also evident in the colour of the muscle tissue. The muscle tissue of residents was a pale off-white colour in comparison to the brightly-coloured orange or pink typical of the muscle tissue of anadromous fish, and the muscle tissue of residents was easy to homogenize into a fine powder. Furthermore, anadromous fish muscle tissue samples that were relatively palecoloured were easily homogenized and were subsequently found to have comparatively low C:N.

**Table 9.** Mean ( $\pm$  SEM) stable isotope and carbon to nitrogen ratios for resident (R) and anadromous (A) Arctic char from four open lake systems in the Cumberland Sound region of the Canadian Arctic, captured from 2002-2008. Isotope signatures and C:N were assessed from untreated (bulk) and lipid extracted (LE) subsamples of white muscle tissue. Change ( $\Delta$ ) was calculated as B - LE.

System	Year	Ecotype		n	δ <sup>13</sup> C		$\delta^{15}N$			C:N			
Qasigiyat	2004	R	Bulk	9	-22.85	±	0.523	9.82	±	0.402	3.60	±	0.043
			LE		-22.61	±	0.537	10.40	±	0.485	3.44	±	0.048
			Δ		0.24	±	0.015	0.57	±	0.082	-0.16	±	0.005
	2004	А	Bulk	11	-21.40	±	0.247	13.72	±	0.164	4.35	±	0.184
			LE		-20.09	±	0.106	14.43	±	0.163	3.49	±	0.034
			Δ		1.31	±	0.141	0.71	±	0.002	-0.85	±	0.150
lqalu-	2004	R	Bulk	15	-26.04	±	0.259	10.00	±	0.243	3.62	±	0.035
gaarjuit			LE		-25.64	±	0.204	9.91	±	0.242	3.42	±	0.042
			Δ		0.40	±	0.055	-0.09	±	0.001	-0.19	±	0.007
	2004	А	Bulk	18	-20.97	±	0.231	13.15	±	0.133	4.65	±	0.240
			LE		-19.51	±	0.083	13.58	±	0.128	3.46	±	0.038
			Δ		1.47	±	0.148	0.43	±	0.005	-1.19	±	0.202
Kipisa	2002	А	Bulk	30	-21.87	±	0.213	14.05	±	0.105	4.98	±	0.307
			LE		-20.50	±	0.070	14.57	±	0.109	3.44	±	0.020
			Δ		1.37	±	0.143	0.52	±	0.004	-1.55	±	0.287
	2003	А	Bulk	21	-21.92	±	0.188	14.20	±	0.087	4.68	±	0.171
			LE		-20.21	±	0.078	14.73	±	0.085	3.40	±	0.017
			Δ		1.71	±	0.110	0.53	±	0.002	-1.28	±	0.154
	2004	А	Bulk	20	-21.39	±	0.163	13.52	±	0.086	4.16	±	0.122
			LE		-19.96	±	0.070	14.17	±	0.100	3.32	±	0.014
			Δ		1.43	±	0.093	0.65	±	0.014	-0.84	±	0.108
Isuituq	2002	А	Bulk	40	-20.36	±	0.106	14.92	±	0.078	4.26	±	0.104
			LE		-19.57	±	0.034	15.31	±	0.074	3.37	±	0.007
			Δ		0.79	±	0.072	0.39	±	0.005	-0.89	±	0.098
	2003	А	Bulk	38	-20.23	±	0.084	14.45	±	0.125	3.93	±	0.067
			LE		-19.65	±	0.038	14.87	±	0.126	3.40	±	0.018
			Δ		0.58	±	0.046	0.42	±	0.001	-0.54	±	0.049
	2004	А	Bulk	20	-20.18	±	0.117	14.01	±	0.122	4.16	±	0.093
			LE		-19.24	±	0.087	14.67	±	0.136	3.32	±	0.010
			Δ		0.94	±	0.030	0.66	±	0.014	-0.83	±	0.084
	2008	А	Bulk	45	-20.41	±	0.103	14.63	±	0.097	4.26	±	0.111
			LE		-19.73	±	0.082	14.80	±	0.113	3.40	±	0.016
			Δ		0.68	±	0.021	0.17	±	0.016	-0.86	±	0.095

**Table 10.** Sulphur stable isotope ratios (mean  $\pm$  SD) for resident and anadromous Arctic char from two open lake systems in the Cumberland Sound region of the Canadian Arctic. Isotope signatures ( $\delta^{34}$ S) were assessed from untreated (bulk) and lipid extracted subsamples of white muscle tissue. Change in  $\delta^{34}$ S (‰) was calculated as B – LE, and were statistically significant (P < 0.05) for both ecotypes.

			Bulk			Lipid Extracted			Change		
Site	Ecotype	n	(‰)		(‰)			(‰)			
Qasigiyat	Resident	9	9.45	±	1.08	8.83	±	1.02	0.62	±	0.33
	Anadromous	10	18.39	±	0.11	17.96	±	0.11	0.44	±	0.46
Iqalugaarjuit	Resident	14	4.71	±	0.40						
	Anadromous	17	17.39	±	0.05						

#### 4.3.1 Lipid Extraction Effects

As expected, lipid extraction resulted in a significant reduction in C:N, from a pooled mean of  $4.28 \pm 0.52$  (bulk) to  $3.40 \pm 0.006$  (LE) (W = -35936, Z = -14.147, *P* < 0.001). The lipid extracted C:N were not significantly different among the ecotypes or sites in Qasigiyat and Iqalugaarjuit (H = 4.411, df = 3, *P* = 0.220). The C:N of anadromous fish following lipid extraction was significantly different among sites (H = 34.716, df = 3, *P* < 0.001), and years (H = 46.955, df = 3, *P* < 0.001), although the coefficient of variation (CV) was reduced for all groups, and the mean CV decreased almost one order of magnitude from 17.0% to 2.7%.

All three stable isotope ratios assessed ( $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S) were significantly higher following lipid extraction, at magnitudes of 0.96 ± 0.048‰ for  $\delta^{13}$ C, 0.42 ± 0.026‰ for  $\delta^{15}$ N, and 0.77 ± 0.097‰ for  $\delta^{34}$ S for pooled data (Z = 12.640, df = 269, *P* < 0.001; Z = 13.635, df = 269, *P* < 0.001, and *t* = 5.672, df = 18, *P* < 0.001, respectively). Likewise, there was significant <sup>13</sup>C-enrichment of the treated samples for both resident (*t* = -3.864, df = 23, *P* < 0.001) and anadromous fish (Z = 13.137, df = 244, *P* < 0.001) (Fig. 25a). Significant enrichment of <sup>15</sup>N was evident for anadromous fish (Z = 12.298, df = 244, P < 0.001), but not for the resident ecotype (Z = 0.686, df = 24, P = 0.502). The  $\delta^{34}$ S of both anadromous (t = 5.690, df = 8, P < 0.001) and resident (t = 3.005, df = 9, P = 0.015) Arctic char was significantly higher following lipid extraction, with  $\Delta \delta^{34}$ S of 0.62‰ and 0.44‰, respectively (Table 10, Fig. 25a).

The CV of pooled data for  $\delta^{13}$ C did not change with sample treatment (7.4% for both bulk and lipid extracted), and there were only slight increases in the CV of  $\delta^{15}$ N (bulk = 10.6% to LE = 10.8%) and  $\delta^{34}$ S (bulk = 0.36% to LE = 0.38%). There was minimal change in the CV following lipid extraction for both resident (bulk = 34.4%, LE = 34.8%) and anadromous fish (bulk = 1.8%, LE = 2.1%).

#### 4.3.2 Effect of Initial Lipid Content

The  $\Delta$ C:N ranged from -7.70 to 0.19, and was negatively correlated to the C:N of the bulk sample (r = 0.994, n = 268, P < 0.001). The magnitude of  $\Delta\delta^{13}$ C ranged from -0.72 to 3.54‰, with lower average  $\Delta\delta^{13}$ C for residents than for anadromous fish (Fig. 25a). The  $\Delta\delta^{13}$ C of pooled data had a strong positive correlation with bulk C:N (C:N<sub>B</sub>; r = 0.829, n = 268, P < 0.001). However, the shape of the relationship was non-linear (Fig. 26): as C:N<sub>B</sub> approached 4.5,  $\Delta\delta^{13}$ C began to level off towards an asymptote of approximately 3.75‰. Below C:N<sub>B</sub> of ≈ 4.5 the relationship between C:N<sub>B</sub> and  $\Delta\delta^{13}$ C was roughly linear, and it should be noted that the C:N<sub>B</sub> of all resident fish was < 4.5.

The  $\Delta \delta^{15}$ N for pooled data was weakly correlated to C:N<sub>B</sub> (r = 0.190, n = 268, P = 0.002). The mean  $\Delta \delta^{15}$ N among groups ranged from -0.09 to 0.71‰, with no significant difference between ecotypes.

The mean  $\delta^{34}$ S ranged from 4.71 ± 0.04‰ for residents to 18.39 ± 0.11‰ for anadromous fish, and following lipid extraction the  $\Delta\delta^{34}$ S of individual samples ranged

from -0.26 to 1.09‰. The  $\Delta\delta^{34}$ S means for each ecotype are given in Table 10. No significant relationship was observed between  $\Delta\delta^{34}$ S and C:N<sub>bulk</sub> (r = 0.311, n = 19, P = 0.195).

# 4.3.3 Effects of Location and Year

Change in  $\delta^{13}$ C and  $\delta^{15}$ N differed between the study sites for the resident ecotype. Residents from Iqalugaarjuit exhibited small but significant increases in both  $\delta^{13}$ C (t = -3.300, df = 14, P = 0.005) and  $\delta^{15}$ N (t = 3.986, df = 14, P = 0.001) after lipid extraction, whereas there were no significant changes detected for either isotope in Qasigiyat residents ( $\delta^{13}$ C, t = -1.942, df = 8, P = 0.088;  $\delta^{15}$ N, t = -1.781, df = 8, P = 0.113) (Fig. 25a).

In contrast, location did not seem to factor into the changes in  $\delta^{13}$ C and  $\delta^{15}$ N for anadromous Arctic char, which consistently exhibited significant differences in carbon and nitrogen isotopes after lipid extraction (Fig. 25b). No differences were detected in C:N,  $\Delta\delta^{13}$ C, or  $\Delta\delta^{15}$ N among years (Fig. 25c).





**Figure 25.** Change  $(\Delta) \pm SD$  (error bars) in  $\delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^{34}S$  and C:N following lipid extraction ( $\Delta =$  lipid extracted – bulk) of Arctic char white muscle tissue, separated by (a) ecotype, including resident (R) and anadromous (A), (b) site of capture, and (c) year of capture.



**Figure 26.** Relationship of bulk carbon-nitrogen ratio (C:N<sub>bulk</sub>) of Arctic char white muscle tissue and the change in carbon stable isotope ratio ( $\Delta\delta^{13}$ C) following lipid extraction (LE), where  $\Delta = LE$  - bulk. Points show data for resident and anadromous ecotypes, and lines represent expected  $\Delta\delta^{13}$ C derived from the lipid-correction models of (a) Post et al. (2007), (b) Kiljunen et al. (2004), and (c) McConnaughey and McRoy (1979).

# 4.3.4 Assessment of Lipid Normalization Models

The four models of lipid normalization were assessed based on comparisons of  $\delta^{13}$ C of bulk tissue to predicted  $\delta^{13}$ C from the models and observed  $\delta^{13}$ C from lipid extraction (Fig. 27). These comparisons were done for each ecotype (i.e. resident and anadromous) separately, as well as for the whole population (i.e. both). Paired *t*-tests revealed significant differences between the observed and predicted  $\delta^{13}$ C for each ecotypes and the population for all models, with the exception of the Post et al. (2007) and Sweeting et al. (2006) models, where there were no differences between observed and model-predicted  $\delta^{13}$ C for the resident ecotype (Table 11).

Linear regression showed strong relationships between observed and predicted  $\delta^{13}$ C of the resident ecotype for all four models, with coefficients of determination  $(R^2)$ ranging from 0.964 to 0.982; however, this relationship was not strong for the anadromous ecotype (Table 11). The Sweeting et al. (2006) model showed the strongest observed-predicted  $\delta^{13}$ C relationship for the anadromous ecotype, with an  $R^2$  of 0.616 (Fig. 27c), followed by the Kiljunen et al (2006) model, with an  $R^2$  of 0.604 (Fig 28d). The strongest relationship at the population level (i.e. both ecotypes) was shown by the Sweeting et al. (2006) model. The McConnaughey and McRoy (1979) model was least effective in predicting  $\delta^{13}$ C and consistently overestimated  $\delta^{13}$ C (Fig. 27a). Modelling efficiency values (EF; Table 11) provided similar results. The Kiljunen et al. (2006) model underestimated  $\delta^{13}$ C and resulted in low modelling efficiency (Fig. 27b). The linear model derived by Post et al. (2007) was found to be fairly accurate at low C:N ratios (< 4.0; Fig. 27), having high EF when applied to the resident ecotype (Table 11). However, the linearity of the relationship between  $\Delta \delta^{13}$ C and bulk tissue C:N values breaks down as C:N increased, and resulted in a negative modelling efficiency and the lowest coefficient of determination for anadromous fish for all models assessed. All four models resulted in negative modelling efficiencies when applied to the anadromous ecotype only.



**Figure 27.** Comparison of observed  $\delta^{13}$ C of lipid-extracted tissue of resident ( $\circ$ ) and anadromous ( $\bullet$ ) ecotypes to expected  $\delta^{13}$ C ( $\delta^{13}$ C') derived from the lipid-correction models of (a) McConnaughey and McRoy (1979), (b) Kiljunen et al. (2004), (c) Post et al. (2007), and (d) Sweeting et al. (2006). The 1:1 line is given for comparison.

**Table 11.** Evaluation of the performance of several proposed lipid-correction models applied to resident (R), anadromous (A), and both ecotypes of Arctic char from the Cumberland Sound region. Model fit was assessed with the linear regression coefficient of determination ( $R^2$ ), modelling efficiency (EF), and paired *t*-tests to compare observed and predicted values of  $\delta^{13}$ C.

Model	Ecotype	$R^2$	EF	t	df	Р
McConnaughev &	R	0.964	0.937	3.943	24	< 0.001
McRoy (1979)	А	0.373	-5.939	-13.147	244	< 0.001
	Both	0.759	0.324	-13.651	268	< 0.001
Kiliunen et al. (2006)	R	0.976	0.762	-13.898	24	< 0.001
	А	0.604	-4.181	13.542	244	< 0.001
	Both	0.951	0.468	14.191	268	< 0.001
Post et al. (2007)	R	0.974	0.971	0.953	24	0.350
	А	0.372	-0.027	-2.602	244	0.009
	Both	0.904	0.897	-2.706	268	0.007
Sweeting et al.	R	0.982	0.982	-0.323	24	0.750
(2006)	А	0.616	-0.080	11.865	244	< 0.001
	Both	0.955	0.894	-15.646	268	< 0.001

# 4.4 Discussion

The results of this study emphasize the need to understand the effects of lipids and lipid treatment before interpreting the results of stable isotope analysis, especially when comparing between and within populations. The <sup>13</sup>C-enrichment following lipid extraction underscores the need to account for lipids when interpreting results of stable isotope analysis for Arctic char, and the changes in  $\delta^{15}$ N and  $\delta^{34}$ S suggest that analyses of these isotopes may need to be conducted from bulk tissue to avoid treatment effects. Although others have reported the effects of lipids and lipid extraction on the  $\delta^{13}$ C and  $\delta^{15}$ N of fish species (Sotiropolous et al. 2004, Mintenbeck et al. 2008), the effect on  $\delta^{34}$ S has not been explicitly tested in fish, and this study demonstrates the importance of addressing lipids in the stable isotope research of partially migratory fish species, in particular between and within populations. The comparison of relatively low and high lipid ecotypes, resident and anadromous Arctic char, respectively, provides a test to evaluate the necessity of extracting lipids in populations with multiple ecotypes. Although C:N may not be directly proportional to lipid content due to species and population specific variability (Fagan et al. 2011), C:N is a good indicator of lipid content in general terms, as C:N and the lipid content of fish muscle tissue is positively associated (Mintenbeck et al. 2008). The ecotypes studied here have widely different lipid content, implied by significantly different C:N and qualitative observations made during sample preparation.

The chloroform-methanol extraction method I used based on the Bligh and Dyer (1959) technique seemed to be generally effective at removing lipids from most of the samples. The ecotypes had a large difference in the C:N of bulk tissue that was successfully standardized by the treatment. Standardization was indicated by the decreased coefficients of variation (CV), similar to the decrease in C:N variation noted by Mintenbeck et al. (2008) for two notothenioid fish species using the same extraction method. However, the significant differences among the C:N of anadromous samples after lipid extraction suggests that full lipid removal was not accomplished. Post et al. (2007) recommended a C:N<sub>LE</sub> of < 3.5 for fish is necessary for unbiased results, and the maximum in the samples analysed here was 3.94; however, C:N seemed sufficiently low, with the upper quartile of samples (75%) with C:N < 3.5. The application of a chloroform-methanol lipid extraction technique has repeatedly been found effective in a range of species and is widely recommended (Mintenbeck et al. 2008, Doucette et al. 2010). Therefore, for lipid-rich fish such as anadromous Arctic char, it may be necessary to conduct more rigorous laboratory procedures to ensure full lipid extraction. Lipid

extractions for high-lipid species likely require a preliminary lipid extraction and grinding sequence, followed by a second sequence conducted on the pre-extracted, preground tissue to ensure all lipid content is removed.

The <sup>13</sup>C-enrichment of tissue samples following the removal of lipids is indicative of lipid bias. In samples with high bulk tissue lipid content, the  $\delta^{13}$ C was artificially low compared to those with low bulk tissue lipid content, owing to the preferential retention of the lighter carbon isotope in lipids (Post et al. 2007). This bias is especially important to consider when interpreting  $\delta^{13}$ C in terms of trophic niche and when comparing among individuals with variable lipid content (Post et al. 2007). Comparisons of  $\delta^{13}$ C between low C:N residents and high C:N anadromous fish within the study systems studied here would produce artificially disparate results due to this lipid bias. Therefore,  $\delta^{13}$ C values must be adjusted or the lipid levels standardized to allow for valid comparisons between these groups.

Sulphur and nitrogen stable isotope ratios ( $\delta^{34}$ S and  $\delta^{15}$ N) were both significantly higher following chemical lipid extraction. This may be the first reported evidence of a lipid treatment effect on the sulphur isotope ratio in fish. A lipid-extraction effect on sulphur was reported by Oppel et al. (2010) in a study of bird egg yolk, and the authors found a  $\delta^{34}$ S increase of 2.3 ± 1.1‰ following chemical lipid extraction using the same Bligh and Dyer (1959) methods as used in this study. The  $\delta^{34}$ S increase reported by Oppel et al (2010) is over four times as large as the mean treatment effect of  $\delta^{34}$ S I found, which is likely due to differences in the composition of egg yolk and fish muscle. In fish, the sulphur-containing amino acids, particularly methionine, are important for protein production and other physiological purposes (Wilson 2002). As speculated by Oppel et

al. (2010), the  ${}^{34}$ S-enrichment observed after chemical extraction could be due to loss of protein – and thereby amino acids – during the extraction process. The mechanism for this is as yet unknown.

In contrast to the findings of Oppel et al. (2010) the average <sup>34</sup>S-depletion found may not constitute an ecologically significant difference, especially if these data are used to identify habitat use where the habitats have widely different  $\delta^{34}S$  (e.g. Fry and Chumcal 2011). For instance, the magnitude of the average  $\delta^{34}S$  difference between the two ecotypes (11.20‰) identified in this study is much larger than the average  $\Delta\delta^{34}S$  increase following lipid extraction (0.53‰). Therefore, the validity of  $\delta^{34}S$  determined from lipid extracted samples would rely on the level of detail required by a particular study. Because current practise is to conduct  $\delta^{34}S$  separately from  $\delta^{13}C$  and  $\delta^{15}N$  (e.g. Barnes and Jennings 2007), it would be preferable to avoid extraction effects altogether by analysing  $\delta^{34}S$  from bulk samples.

The enrichment effect of lipid extraction on  $\delta^{15}$ N observed here corresponds to published reports. Post et al (2007) found a difference of 0.25‰ for  $\delta^{15}$ N between treated and untreated samples; Mintenbeck et al. (2008) found even higher differences, with averages of 1.35‰ and 1.65‰ in two fish species. My results show an average increase in  $\delta^{15}$ N of 0.42‰ following lipid extraction, which falls well within this range. Enrichment of <sup>15</sup>N has been attributed to the leaching of nitrogen-containing proteins during the lipid extraction procedure, particularly proteins that are associated with structural lipids (Sotiropolous et al. 2004). Despite evidence supporting this hypothesis (e.g. Mintenbeck et al. 2008), it seems that the precise mechanisms involved in this nitrogen "leak" have yet to be defined or tested.

The nitrogen stable isotope ratio is commonly used in numerous applications of ecosystem – especially food-web – studies, due in part to the fact that the isotope is fractionated predictably (by 3-4‰) as it is transferred across trophic levels. In addition, with current technology  $\delta^{13}$ C and  $\delta^{15}$ N are usually analysed simultaneously. This study provides further evidence that sample treatment must be carefully planned prior to analysis to avoid skewing results, especially when results are applied to estimate trophic positioning and input into mixing models.

Anadromous and resident Arctic char differed in the magnitude of the  $\delta^{13}$ C change following lipid extraction, which was related to the initial lipid content (i.e. using C:N as a proxy) of the tissue. This finding illustrates how lipid effects on  $\delta^{13}$ C can bias results even within a species. The change in  $\delta^{13}$ C was smaller for residents than for anadromous Arctic char, which can be attributed to the lower bulk tissue lipid content of residents. Despite bulk tissue C:N values of < 4.0, resident  $\delta^{13}$ C was affected by lipid extraction, suggesting that extraction may be warranted even for relatively low-lipid samples.

The location-specific differences within the resident ecotype observed for both  $\delta^{13}$ C and  $\delta^{15}$ N may reflect differences in the trophic resources of the specific lakes in question. Because lakes are isolated from one another, it is not surprising that some differences exist among the resident ecotypes, whereas anadromous fish, which occupy the more homogenous marine environment, are more similar to one another, utilizing similar marine resources (Fry and Scherr 1984; see Section 2.3.2). The differences in bulk C:N between ecotypes and the changes in  $\delta^{13}$ C following lipid extraction underscore the importance of accounting for lipid differences when using stable isotopes to assess intraspecific differences in resource use, where wide variations in lipid content within a

single population can strongly bias interpretations of trophic niches.

None of the arithmetic lipid normalization models I tested provided accurate predictions of  $\delta^{13}$ C for lipid extracted Arctic char muscle tissue at the population level. Significant differences have been observed between observed and predicted  $\delta^{13}$ C in other studies using the models of McConnaughey and McRoy (1979), Post et al. (2007), and Sweeting et al. (2006), such as those reported by Doucette et al. (2010) for cormorants (*Phalacrocorax auritus*). Similarly, in a study of numerous species of freshwater and marine fishes, Kiljunen et al. (2006) found that the McConnaughey and McRoy (1979) model-predicted  $\delta^{13}$ C did not accurately represent lipid extracted  $\delta^{13}$ C values.

In the present study, the effectiveness of the models in predicting  $\delta^{13}C$  differed with ecotype. The models suggested by Post et al. (2007) and Sweeting et al. (2006) sufficiently predicted  $\delta^{13}C$  for the resident ecotype, which must be considered with reference to the distinct difference in bulk C:N between the ecotypes. Resident fish were found to have low C:N ratios that changed very little following lipid extraction, and therefore lipids may not have a large effect on the  $\delta^{13}C$  of these fish. For the samples that lipids would actually create a bias in  $\delta^{13}C$  – the anadromous fish, which had a C:N of up to 11.53 – none of the normalization models were sufficient.

For the fish with higher C:N (i.e. anadromous), the McConnaughey and McRoy (1979) model under-estimated  $\delta^{13}$ C, whereas the Kiljunen et al. (2007) model overestimated  $\delta^{13}$ C. The Kiljunen et al. (2006) model was proposed as a revised version of the McConnaughey and McRoy (1979) model that was supposed to better reflect fish species from both marine and fresh water by addressing the underestimated  $\delta^{13}$ C, especially at high C:N. The results of my study suggest that these models may not be applicable to the wide range of species that the authors suggest; rather, validation for a potential study species is warranted before these models are applied.

As suggested by the non-significant difference between observed and predicted  $\delta^{13}C$ for resident fish, the linear model derived by Post et al. (2007) was found to be fairly accurate at low C:N ratios (< 4.0), as shown by its high EF when applied to the resident ecotype. The linearity of the Post et al. (2007) model, which appeared to be applicable when lipid content was low (i.e. C:N < 4), becomes ill-fitting with high C:N, as observed with the anadromous ecotype. This asymptotic relationship was reported by Logan et al. (2008), who suggest that fitting data to a linear model would not be appropriate at all C:N levels. The shape of the relationship between  $\Delta \delta^{13}$ C and C:N<sub>bulk</sub> at the population level better reflected the models proposed by McConnaughey and McRoy (1979), and Kiljunen et al. (2006), wherein  $\Delta \delta^{13}$ C approached an asymptote, theoretically defined as the discrimination factor between pure lipid and lipid-free tissue (Kiljunen et al. 2006). Yet these models were still ineffective at capturing the true relationship of  $\Delta \delta^{13}$ C and C:N<sub>bulk</sub> in this species, as shown by their low modelling efficiencies. These models utilize and attempt to encompass the lipid composition patterns of a broad range of species, yet there is much variation between and even within species (Fagan et al. 2011). Therefore, unless model is fitted to the exact population – and even ecotype – under study, lipid normalization should not be applied. Indeed, the low predictive capacity of the models tested here suggests that modifying or creating a model using species-specific (or as in this case, ecotype-specific) parameters is necessary to reliably use mathematical lipid normalization. As proposed by Logan et al. (2008), a sample subset could be assessed for C:N<sub>bulk</sub>, and bulk and lipid extracted values of  $\delta^{13}$ C for generation of a model that could

then be applied to the rest of the data. This would decrease the time and effort involved in lipid extraction and dual analysis of the samples.

In conclusion, lipids can have a significant effect on the  $\delta^{13}$ C of fish muscle samples that must be accounted for prior to further analysis. The most reliable method of accounting for the effect of lipids is to remove the lipids from the sample; however, this chemical extraction alters the amounts of other isotopic components, specifically  $\delta^{15}$ N and to a lesser extent  $\delta^{34}$ S. Therefore, ideally all samples should be analysed in both bulk and lipid extracted forms. Lipid normalization models have been proposed as a way to avoid time-consuming lipid extractions by predicting the lipid-free  $\delta^{13}$ C from bulk samples. Yet the current models are too generalized to fully capture the natural patterns of carbon stable isotope ratios, especially for fish with high lipid content such as the anadromous ecotype of Arctic char. In addition, differences in the relationship between C:N and  $\delta^{13}$ C at an intra-population level can be present. Therefore, future attempts at determining appropriate model equations and parameters must validate these relationships not only amongst species, but among smaller ecological units.

# **General Conclusions**

The study of resource partitioning in partially migratory populations is essential for understanding the processes relating to adaptive divergence and ultimately speciation (Knudsen et al. 2006). In addition, both direct and indirect anthropogenic impacts, including climate change, are pressuring this, as well as other Arctic aquatic ecosystems. Thus, the Arctic char of the Cumberland Sound region presented a unique opportunity for trophic ecology research, owing to the presence of distinct migratory ecotypes and due to the recent invasion of a forage fish.

This study provides insight into the trophic ecology of Arctic char using a multiindicator approach. My data support previously proposed trophic niche differences between resident and anadromous ecotypes, and among mature anadromous, mature resident, and immature Arctic char, evident in both stable isotope and fatty acid profiles. This is the first application of fatty acids in comparing resident and anadromous Arctic char ecotypes. The work presented here provides evidence for use of the inter-tidal zone by the resident ecotype in Qasigiyat, implying low productivity of this system, and suggesting a strategy to maximize access to resources by residents, and avoidance of intraspecific competition.

Arctic marine systems are experiencing significant change. Cumberland Sound is now home to capelin, providing new prey for marine predators. Whereas historically, the diet composition of Arctic char in this region was dominated by zooplankton (primarily amphipods), their diets in 2011 were dominated by capelin, a shift that has occurred within the past six years. Arctic char foraging is known to be opportunistic, a fitness advantage in variable, patchy environments such as those found in polar marine systems,

and thus Arctic char diets often track prey abundances or densities in their environments. I suggest that this shift in Arctic char diets is due to the combined effect of increased capelin availability and decreased zooplankton availability in Cumberland Sound. The observed change in diet was not accompanied by significant change in  $\delta^{15}$ N, which may be due to a lack of difference in trophic level between past and present prey items, although discrete trophic levels could not be determined. The observed temporal  $\delta^{13}$ C change may be diet related, or may stem from changes in baseline  $\delta^{13}$ C owing to oceanographic conditions. Condition was variable for Arctic char populations among years, but a significant trend was not observed; this result implies that Arctic char are capable of meeting their energy requirements during the summer feeding season by feeding on capelin. However, the increased length-at-age observed for fish in years subsequent to 2005 implies better overall growth for fish in years following the possible date of capelin inclusion in Arctic char diets, leading me to propose that the quality of capelin as a prey source is relatively equal to that of historical prey items (i.e. zooplankton). A longer time series is essential to further assess the effects of this diet switch on Cumberland Sound Arctic char populations, in addition to collection of prey stable isotope data that includes baseline stable isotope ratios.

Finally, the effects of lipids and lipid extraction on stable isotope ratios were investigated to provide validation for prior research methods. In accordance with the literature, I found lipid effects on  $\delta^{13}$ C and lipid-extraction effects on  $\delta^{15}$ N and  $\delta^{34}$ S. This study provided the first evidence of a lipid-extraction effect on fish  $\delta^{34}$ S; however, the level of effect was lower than the differences usually assessed in the literature, indicating an effect that may not be significant when used in biological or ecosystem studies. Arithmetic lipid-correction models were assessed and applied to fish populations with high- and low-lipid components (anadromous and resident Arctic char, respectively) to determine if the models could be appropriate substitutes for lipid extraction in estimating  $\delta^{13}$ C. Results showed that models did not provide adequate estimates of  $\delta^{13}$ C, especially when applied to high-lipid samples. It was thus recommended that unless populationspecific model validations are performed (and sometimes, as found here, at a subpopulation-specific),  $\delta^{13}$ C should be analysed from lipid extracted samples whereas  $\delta^{15}$ N and  $\delta^{34}$ S be analysed from bulk samples.

Arctic char represent a significant cultural, subsistence, and economic resource in this region, with Inuit subsistence and commercial capture fisheries relying on the health of Cumberland Sound Arctic char populations (DFO 2005, 2010). This thesis has assessed the trophic ecology of Arctic char in terms of both intraspecific variability and long-term temporal trends, with implications for fisheries managers in this area, contributing to effective conservation and ecosystem-based management.

## Future Directions

Although there is relatively substantial work being done on marine mammals (beluga: Marcoux et al. 2012; bowhead whale: Pomerleau et al. 2011), and higher trophic level fish (Greenland shark *Somniosus microcephalus*: Fisk et al. 2002; Greenland halibut: Dennard et al. 2009, Arctic char: DFO 2005, 2010), there is still a dearth of information on the lower trophic levels within Cumberland Sound (but see McMeans et al. 2012). Because Arctic char diets generally reflect the prey composition of their environment (Rikardsen et al. 2007), the present research highlights food web changes in Cumberland Sound that are likely having an effect on many ecosystem components. A

consistent long-term sampling program for zooplankton and forage fish would be ideal to fully understand the trophic dynamics within this system. This type of sampling program is particularly relevant for Arctic marine systems, such as this one, that are most likely undergoing significant changes in food web structure. Marine mammals and higher trophic level fish are both economically and culturally important in the Cumberland Sound region, and zooplankton and forage fish are the prey base for these organisms, either directly or indirectly (i.e. prey of prey). Thus having a grasp on the dynamics of the prey base would be important for informing future management decisions regarding higher trophic level marine predators.

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## Appendix A Coding for ANCOVA

Coding for analysis of covariance in R (R Core Development Team 2008), where  $\delta^{15}$ N was the independent continuous variable, year was the categorical variable, and fork length (L<sub>F</sub>) was the covariate. The example here is given with Kipisa data, and follows

```
Crawley (2007).
```

```
#load data and attach headings
attach(pg004.si)
names(pg004.si)
pg004.si$year.k<-factor(pg004.si$year.k)</pre>
year.k<-factor(pq004.si$year.k)</pre>
#fit a linear model
ancova<-lm(d15n.k~year.k*lf.k)</pre>
#remove the interaction term if not significant
ancova2<-update(ancova,~.-year.k:lf.k)</pre>
#compare the original and simplified models
anova (ancova, ancova2)
#remove the other variable and compare the fits
ancova3<-update(ancova2,~.-year.k)</pre>
anova (ancova2, ancova3)
#retreive adjusted means
adjmeans.k<-effect("year.k", ancova2, se=TRUE)</pre>
summary(adjmeans.k)
#conducting pairwise comparisons using best model
ancova22<-aov(d15n.k~year.k+lf.k)</pre>
TukeyHSD(ancova22, "year.k", ordered=FALSE, conf.level=0.95)
```

### Appendix B Coding for VBGM

Coding for von Bertalanffy growth model using non-linear least squares (nls) in the vegan package in R (R Core Development Team 2008), using Kipisa data from the first period (1998-2001).

```
#load data and attach headings
attach(pg004g)
pg004g$year.k<-factor(pg004g$year.k)
pq004q$fish.id.k<-factor(pq004q$fish.id.k)</pre>
#subset the dataset for group comparisons
pg004.BC<-subset(pg004g,group.k=="BC")
pq004.DC<-subset(pq004q,group.k=="DC")
pg004.PC<-subset(pg004g,group.k=="PC")
#determine starting values for input into VBGM
sv.kBC<-vbStarts(lf.k~age.k,data=pq004.BC,plot=T)</pre>
unlist(sv.kBC)
sv.kDC<-vbStarts(lf.k~age.k,data=pg004.DC,plot=T)</pre>
unlist(sv.kDC)
sv.kPC<-vbStarts(lf.k~age.k,data=pq004.PC,plot=T)</pre>
unlist(sv.kPC)
#general VBGM fitting with nls()
vb.kBC<-lf.k~Linf*(1-exp(-K*(age.k-t0)))</pre>
fit.kBC<-nls(vb.kBC,data=pq004.BC,start=sv.kBC)</pre>
vb.kDC < -lf.k \sim Linf * (1 - exp(-K*(age.k-t0)))
fit.kDC<-nls(vb.kDC, data=pg004.DC, start=sv.kDC)</pre>
vb.kPC<-lf.k~Linf*(1-exp(-K*(age.k-t0)))</pre>
fit.kPC<-nls(vb.kPC,data=pg004.PC,start=sv.kPC)</pre>
#visual of fitted model
fitPlot(fit.kBC,main="",xlab="Age (years)",ylab="Fork
Length (mm)")
#summary results of model fit
overview(fit.kBC)
```

```
#finding Confidence Intervals for the parameters with
bootstrap methods
boot.kBC<-nlsBoot(fit.kBC,niter=200)
confint(boot.kBC,plot=T)
```

```
#predicted lengths at a given age
kBC.age10<-data.frame(age.k=10)</pre>
predict(fit.kBC,kBC.age10)
ests<-boot.kBC$coefboot
pv<-ests[,"Linf"]*(1-exp(-ests[,"K"]*(8-ests[,"t0"])))</pre>
quantile(pv,c(0.025,0.975)) #gives mean length of all fish
of the assessed age
#Create fitted line plot with confidence bounds for mean
#length-at-age
par(mfrow=c(1,1))
ages2plot<-0:30</pre>
fitPlot(fit.kBC,xlab="Age (years)",ylab="Fork Length
(mm)", xlim=range(ages2plot), main="")
LCI<-UCI<-numeric(length(ages2plot))</pre>
for (i in 1:length(ages2plot)) {
pv <- ests[,"Linf"]*(1-exp(-ests[,"K"]*(ages2plot[i]-</pre>
ests[,"t0"])))
LCI[i] <- quantile(pv, 0.025)</pre>
UCI[i] <- quantile(pv, 0.975)</pre>
}
lines(UCI~ages2plot,type="1",col="blue",lwd=2,lty=2)
lines(LCI~ages2plot,type="1",col="blue",lwd=2,lty=2)
#produce scatterplots for bootstrapped values for parameter
#pairs
plot(boot.kBC)
#checking assumptions
residPlot(fit.kBC)
hist(residuals(fit.kBC),main="")
```

# Appendix C Coding for Likelihood Ratio Tests

R coding for likelihood ratio tests for comparing von Bertalanffy growth curves using the fishmethods package (*sensu* Kimura 1980; Cerrato 1990). Example is given with Kipisa data comparing the first two periods (1998-2001 and 2002-2005).

```
#load data and attach headings
attach(pg004g1)
#likelihood ratio test for comparing two VBGM
vblrt(len=pg004g1$lf.k,age=pg004g1$age.k,group=pg004g1$grou
p.k,error=1, select=1)
```

#### Appendix D Results of Likelihood Ratio Tests for VBGM

Statistical results of likelihood ratio tests of von Bertalanffy growth model parameters applied to pairwise combinations of periods sampled within each study system (Kipisa and Isuituq). H<sub>0</sub> refers to the general model, wherein all parameters are different between the models. H<sub>1</sub> to H<sub>4</sub> refer to the alternative models, wherein at least one parameter is in common between the models: H<sub>1</sub>:  $L_{\infty 1} = L_{\infty 2}$ ; H<sub>2</sub>: K<sub>1</sub> = K<sub>2</sub>; H<sub>3</sub>: t<sub>01</sub>= t<sub>02</sub>; H<sub>4</sub>:  $L_{\infty 1} = L_{\infty 2}$ , K<sub>1</sub> = K<sub>2</sub>, t<sub>01</sub>= t<sub>02</sub>. The general model is compared to each sub-model using the residual sum of squares and tested for significance with the chi-squared test statistic (Kimura 1980). Significant differences are indicated with an asterisk (\*).

Kipisa				Isuituq			
Test	X <sup>2</sup>	df	P-value	Test	χ <sup>2</sup>	df	P-value
1998-2001	vs 200	2-2005	5	2002-2005 v	rs 2006-20	09	
$H_0 vs H_1$	0.11	1	0.740	$H_0 vs H_1$	0.31	1	0.580
$H_0 vs H_2$	0.68	1	0.410	$H_0 vs H_2$	3.09	1	0.079
$H_0 vs H_3$	0.75	1	0.386	$H_0 vs H_3$	8.90	1	0.003*
$H_0 vs H_4$	5.13	3	0.163	$H_0 vs H_4$	56.54	3	< 0.001*
1998-2001	vs 201	1		2002-2005 v	rs 2011		
$H_0 vs H_1$	12.67	1	< 0.001*	$H_0 vs H_1$	< 0.01	1	1.000
$H_0 vs H_2$	12.11	1	0.001*	$H_0 vs H_2$	0.79	1	0.374
$H_0 vs H_3$	3.97	1	0.046*	$H_0 vs H_3$	6.92	1	0.009*
$H_0 vs H_4$	61.75	3	< 0.001*	$H_0 vs H_4$	23.42	3	< 0.001*
2002-2005	5 vs 201	1		2006-2009 v	rs 2011		
$H_0 vs H_1$	7.89	1	0.005*	$H_0 vs H_1$	0.50	1	0.480
$H_0 vs H_2$	6.49	1	0.011*	$H_0 vs H_2$	0.91	1	0.340
$H_0 vs H_3$	1.23	1	0.267	$H_0 vs H_3$	< 0.01	1	1.000
$H_0$ vs $H_4$	28.50	3	< 0.001*	$H_0$ vs $H_4$	58.60	3	< 0.001*

**Appendix E** Reference photographs of Arctic char ecotypes



**Figure 28.** Arctic char classified as the resident ecotype, captured from Iqalugaarjuit in September 2011.



Figure 29. Immature Arctic char captured from Iqalugaarjuit in September 2011.



**Figure 30.** Arctic char captured from Iqalugaarjuit in September 2011. The individual at the top of the picture was a mature resident; the other two were classified as immature.



**Figure 31.** Mature female Arctic char classified as the anadromous ecotype, captured from Iqalugaarjuit in September 2011.



**Figure 32.** Arctic char captured from Qasigiyat in September 2011. The individual at the top of the picture was immature; the individual at the bottom was a mature resident.



**Figure 33.** Mature male Arctic char captured from Iqalugaarjuit in September 2011, classified as the resident (top) and anadromous (bottom) ecotypes.

Appendix F	Raw	data:	field	collections

Location	Net Lift date (DD/MM/YR)	Sample #	Fork Length (mm)	Weight (g)	Sex (Male/ Female)	Maturity Status	Gonad Weight (g)	Stomach Preserved (Y/N)	Age (From Otoliths)	Ecotype Group
Isuitug	16/08/11	001	633	3270	F	R	48.0	Ŷ	16	A .
Isuitug	16/08/11	002	625	2572	F	I	17.0	N	15	Α
Isuitug	16/08/11	003	422	1196	Μ	l	0.5	Y	7	Α
Isuitug	16/08/11	004	391	617	Μ	I	<0.5	Y	7	Α
Isuituq	16/08/11	005	668	4055	F	R	89.0	N	15	Α
Isuituq	16/08/11	006	550	1424	F	R	17.0	N	22	Α
Isuituq	16/08/11	007	629	2975	М	R	6.0	N	10	Α
Isuituq	16/08/11	008	432	985	М	I	0.5	N	10	Α
Isuituq	16/08/11	009	496	1523	F	I	11.0	N	11	Α
Isuituq	16/08/11	010	648	3294	F	М	132.0	Y	16	Α
Isuituq	16/08/11	011	597	2565	F	I	22.5	Y	17	Α
Isuituq	16/08/11	012	645	2798	М	R	8.5	N		Α
Isuituq	16/08/11	013	169	46	F	l	<0.5	Y	4	Α
Isuituq	16/08/11	014	185	61	F	I	<0.5	Y	4	Α
Isuituq	16/08/11	015	621	2683	F	R	14.0	Y	14	A
Isuituq	16/08/11	016	377	578	М	I	0.5	N	8	Α
Isuituq	16/08/11	017	401	707	Μ	I	0.5	Y	7	Α
Isuituq	16/08/11	018	544	1999	F	I	13.0	Y	12	Α
Isuituq	16/08/11	019	556	2184	F	I	14.5	Y	13	Α
Isuituq	16/08/11	020	413	743	Μ	I	<0.5	N	7	Α
Isuituq	16/08/11	021	435	772	Μ	I	<0.5	Y	7	Α
Isuituq	16/08/11	022	411	809	М	l	0.5	Y	8	Α
Isuituq	16/08/11	023	412	774	М	I	<0.5	Y	7	Α
Isuituq	16/08/11	024	390	659	М	I	<0.5	Y	7	Α
Isuituq	16/08/11	025	554	1990	М	R	2.0	Ν	8	Α
Isuituq	16/08/11	026	654	2724	М	R	6.0	Ν	13	Α
Isuituq	16/08/11	027	523	1410	М	R	2.5	Ν	10	Α
Isuituq	16/08/11	028	485	1292	М	I	0.5	Y	9	Α
Isuituq	16/08/11	029	394	691	М	I	<0.5	N	7	А
Isuituq	16/08/11	030	615	2551	F	R	13.0	Ν	16	А
Isuituq	16/08/11	031	495	1264	М	R	1.0	Ν	9	Α
Isuituq	16/08/11	032	550	1961	F	I	17.0	Y	13	А
Isuituq	16/08/11	033	374	550	F	I	2.0	Y	8	Α
Isuituq	16/08/11	034	372	558	F	I	1.5	Y	6	Α
Isuituq	16/08/11	035	444	371	F	I	3.5	Y	7	Α
Isuituq	16/08/11	036	494	1392	М	R	2.0	N	10	Α

Isuituq	16/08/11	037	420	1000	F	М	30.0	N	11	A
 Isuituq	16/08/11	038	644	3209	F	R	23.0	Ν	15	Α
lsuituq	. 16/08/11	039	585	2464	М	R	205.0	Y	13	Α
Isuituq	16/08/11	040	520	1385	F	I	6.0	Y	9	А
Isuituq	16/08/11	041	671	3315	М	R	7.0	Ν	14	Α
Isuituq	16/08/11	042	510	1351	М	l	35.0	Y	10	Α
Isuituq	16/08/11	043	512	1662	F	l	1.0	N	10	А
Isuituq	16/08/11	044	719	4426	Μ	R	9.0	Y	15	А
 Isuituq	16/08/11	045	182	60	U	l	<0.5	Y	5	Α
 Isuituq	16/08/11	046	183	63	F	l	<0.5	Y	4	A
 Isuituq	16/08/11	047	180	58	М	I	<0.5	Y	5	A
 Isuituq	16/08/11	048	201	89	M	l	<0.5	Y	5	A
 Isuituq	16/08/11	049	590	2365	M	R	5.5	N	11	A
 Isuituq	16/08/11	050	405	657	F	l	1.0	N	7	Α
 Isuituq	16/08/11	051	465	1043	Μ	l	0.5	N	12	Α
 Isuituq	16/08/11	052	477	1227	F	l	9.0	Y	10	A
 Isuituq	16/08/11	053	516	1624	F	I	9.5	Y	13	A
 Isuituq	16/08/11	054	355	511	М	l	0.5	Y	7	Α
 Isuituq	16/08/11	055	405	737	F	М	31.0	Ν	9	Α
 Isuituq	16/08/11	056	557	1737	Μ	R	1.5	N	14	Α
 Isuituq	16/08/11	057	704	3429	М	R	11.0	Y	14	A
 Isuituq	16/08/11	058	685	3565	М	Μ	52.0	Y	20	Α
 Isuituq	16/08/11	059	607	2414	F	R	16.0	N	16	Α
 Isuituq	16/08/11	060	534	1973	F	R	13.0	Y	10	Α
 Isuituq	16/08/11	061	379	599	М	l	<.5	Y	9	Α
 Isuituq	16/08/11	062	444	847	М	l	0.5	N	10	Α
 Isuitug	16/08/11	063	403	667	F	l	1.5	Y	8	A
 Isuituq	16/08/11	064	410	740	F	l	2.5	Y	9	Α
 Isuituq	16/08/11	065	692	3603	Μ	Μ	67.0	Y	16	Α
 Isuitug	16/08/11	066	383	567	F	l	1.5	N	7	Α
 Isuitug	16/08/11	067	399	618	F	l	3.5	Y	8	Α
 Isuitug	16/08/11	068	553	2034	Μ	R	1.0	Y	10	Α
 Isuitua	16/08/11	069	545	1633	Μ	R	2.5	N	11	Α
 Isuituq	16/08/11	070	582	2599	F	R	11.5	Y	13	Α
 Isuitug	16/08/11	071	533	1477	Μ	l	1.0	N	8	Α
 Isuituq	16/08/11	072	494	1450	М	l	0.5	N	9	Α
 Isuitug	16/08/11	073	402	685	Μ	l	0.5	N	6	Α
 Isuitug	16/08/11	074	485	1361	Μ	l	1.0	Y	9	Α
 Isuitua	16/08/11	075	642	2962	F	R	25.5	N	15	Α
 Isuitua	16/08/11	076	611	2790	F	R	23.5	N	16	Α
 Isuitua	16/08/11	077	450	941	F	<u> </u>	3.5	N	9	Α
 Isuitua	16/08/11	078	481	1211	F	M	86.0	N	10	A
 Isuitug	16/08/11	079	436	872	F	<u> </u>	4.0	Y	8	Α
 Isuituq	16/08/11	080	364	496	M	I	0.5	N	7	Α

Isuituq	16/08/11	081	406	731	F	I	2.5	N	8	A
Isuituq	16/08/11	082	304	294	F	I	1.0	Y	6	A
lsuituq	16/08/11	083	449	942	М	I	0.5	Ν	10	Α
 Isuituq	16/08/11	084	390	565	F	l	1.5	N	8	Α
Isuituq	16/08/11	085	400	861	М	l	0.5	Ν	9	A
Isuituq	17/08/11	086	583	2589	F	R	13.5	Y	13	А
Isuituq	17/08/11	087	588	2328	М	R	0.5	Y	12	Α
 Isuituq	17/08/11	088	619	2712	F	R	20.0	N	13	Α
Isuituq	17/08/11	089	652	2554	F	R	25.0	N	17	Α
Isuituq	17/08/11	090	465	1058	F	I	5.0	Y	8	Α
 Isuituq	17/08/11	091	352	494	М	I	0.5	N	7	Α
 Isuituq	17/08/11	092	650	3137	М	R	6.5	Y	15	Α
 Isuituq	17/08/11	093	480	1359	F	l	4.5	Y	8	Α
 Isuituq	17/08/11	094	594	2392	М	R	2.0	Y	10	Α
 Isuituq	17/08/11	095	456	1336	F	l	7.5	N	9	Α
 Isuituq	17/08/11	096	191	80	М	l	<.5	N	4	Α
 Isuituq	17/08/11	097	405	625	Μ	l	0.5	N	9	Α
 Isuituq	17/08/11	098	397	747	М	l	0.5	Y	7	Α
 Isuitug	17/08/11	099	452	1050	F		5.0	Y	10	Α
 Isuituq	17/08/11	100	455	950	М	l	0.5	N	12	Α
 Isuituq	17/08/11	101	704	3755	М	R	6.0	N	18	Α
 Isuituq	17/08/11	102	458	963	F	l	6.0	Y	10	Α
 Isuituq	17/08/11	103	505	1357	М	l	1.5	Y	12	Α
 Isuituq	17/08/11	104	537	1207	F	R	14.0	N	18	Α
 Isuituq	17/08/11	105	710	2588	М	R	4.5	N	15	Α
 Isuitug	17/08/11	106	180	51	F		<.5	N	5	Α
 Isuituq	17/08/11	107	184	60	F	l	<.5	N	7	Α
 Isuituq	17/08/11	108	515	1818	F	R	11.5	Y	12	Α
 Isuitug	17/08/11	109	635	2893	F	R	26.0	Y	18	Α
 Isuitug	17/08/11	110	662	2950	Μ	R	9.0	N	18	Α
 Isuitug	17/08/11	111	405	650	Μ	l	<.5	Y	8	Α
 Isuitug	17/08/11	112	602	2711	М	R	3.0	Y	15	Α
 Isuitug	17/08/11	113	709	4469	Μ	R	8.5	Y	14	A
 Isuitug	17/08/11	114	593	2483	F	R	19.5	N	12	Α
 Isuitug	17/08/11	115	409	787	F		3.0	N	8	Α
 Isuitug	17/08/11	116	589	2411	F	R	45.0	Y	13	Α
 Isuitug	17/08/11	117	607	2969	Μ	R	2.0	Y	12	Α
 Isuitua	17/08/11	118	566	1694	F	R	1.0	Y	16	Α
 Isuitua	17/08/11	119	634	2926	Μ	R	2.5	Y	13	Α
 Isuitua	17/08/11	120	525	1462	Μ	R	1.5	N	9	Α
 Isuitua	17/08/11	121	422	804	F	R	78.0	N	8	Α
 Isuitua	17/08/11	122	433	823	M		0.5	Y	8	A
 Isuitua	17/08/11	123	643	2775	M	R	5.5	N	14	A
 Isuitua	17/08/11	124	565	1547	F	R	12.0	Y	14	Α
 				-			-			

I	suituq	17/08/11	125	570	2360	Μ	R	3.5	Ν	10	A
I	suituq	17/08/11	126	524	1819	Μ	R	1.5	Y	9	Α
l	suituq	17/08/11	127	545	1709	F	R	8.5	Ν	11	Α
l	suituq	17/08/11	128	515	1672	F	R	15.5	Y	11	Α
I	suituq	17/08/11	129	391	635	Μ	l	<.5	Ν	9	Α
l	suituq	17/08/11	130	495	1359	F	R	8.5	Ν	9	А
I	suituq	17/08/11	131	591	1821	F	Μ	53.5	Ν	14	Α
I	suituq	17/08/11	132	390	618	Μ	l	0.5	Y	8	Α
I	suituq	17/08/11	133	432	851	F		3.5	Ν	8	Α
I	suituq	17/08/11	134	625	2875	F	R	21.5	Y	16	Α
I	suituq	17/08/11	135	465	1119	Μ	I	0.5	Ν	10	Α
I	suituq	17/08/11	136	545	1996	F	R	11.0	Ν	14	Α
I	suituq	17/08/11	137	417	782	F		3.5	Ν	8	Α
l	suituq	17/08/11	138	652	2375	Μ	R	5.0	Y	15	А
l	suituq	17/08/11	139	415	710	Μ		0.5	Y	6	А
l	suituq	17/08/11	140	525	1505	Μ	R	2.5	Y	13	А
l	suituq	17/08/11	141	646	2742	F	R	40.5	Y	19	А
l	suituq	17/08/11	142	180	56	F		<.5	Y	3	А
I	suituq	17/08/11	143	376	549	Μ	I	<.5	Y	7	Α
I	suituq	17/08/11	144	345	420	F	I	1.5	Ν	7	Α
<u> </u>	suituq	17/08/11	145	311	315	Μ		<.5	Ν	6	Α
	suituq	17/08/11	146	535	1705	Μ	R	3.5	Ν	14	Α
	suituq	17/08/11	147	433	765	F		4.0	Ν	9	Α
<u> </u>	suituq	17/08/11	148	405	735	F		3.0	Y	8	Α
<u> </u>	suituq	17/08/11	149	481	1270	F	<u> </u>	6.0	N	12	Α
<u> </u>	suituq	17/08/11	150	509	1398	Μ	<u> </u>	0.5	Y	9	Α
<u> </u>	suituq	17/08/11	151	429	854	Μ	<u> </u>	<.5	Y	8	Α
	suituq	17/08/11	152	362	482	Μ	<u> </u>	<.5	N	9	Α
<u> </u>	suituq	17/08/11	153	401	745	F		2.0	Ν	7	Α
<u> </u>	suituq	17/08/11	154	507	1451	F	R	7.5	Y	10	Α
	suituq	17/08/11	155	574	2151	F	R	11.0	Ν	15	Α
<u> </u>	suituq	17/08/11	156	290	251	Μ	<u> </u>	<.5	N	6	Α
<u> </u>	suituq	17/08/11	157	614	2413	F	R	24.5	N	12	Α
I	suituq	17/08/11	158	681	4012	Μ	Μ	204.5	Ν	16	Α
<u> </u>	suituq	17/08/11	159	580	2339	F	Μ	263.0	N	19	Α
I	suituq	17/08/11	160	392	705	Μ	I	<.5	Y	7	Α
l	suituq	17/08/11	161	601	2210	Μ	R	2.5	Y	13	Α
<u> </u>	suituq	17/08/11	162	480	1257	Μ		0.5	Y	9	Α
<u> </u>	suituq	17/08/11	163	508	1504	Μ	R	1.0	Y	10	Α
<u> </u>	suituq	17/08/11	164	505	1229	F		7.0	Ν	9	Α
<u> </u>	suituq	17/08/11	165	487	1372	Μ	R	1.5	Y	9	Α
I	suituq	17/08/11	166	371	548	Μ		0.5	Υ	6	Α
I	suituq	18/08/11	167	450	1008	F		3.5	Ν	11	Α
I	suituq	18/08/11	168	365	560	F	I	1.5	Y	7	A

Isultuq         18/06/11         170         320         340         M         I         12.5         N         15         A           Isultuq         18/06/11         171         640         3035         F         R         44.0         Y         19         A           Isultuq         18/06/11         172         570         2661         M         R         1.5         N         10         A           Isultuq         18/06/11         173         561         1914         F         R         6.0         Y         17         A           Isultuq         18/06/11         176         616         2666         F         R         12.0         Y         13         A           Isultuq         18/06/11         176         534         1634         M         R         0.5         N         11         A           Isultuq         18/06/11         176         534         1634         M         R         0.5         N         11         A           Isultuq         18/06/11         160         652         3492         F         R         120.0         N         20         A           Isultuq <th></th>											
	 Isuituq	18/08/11	169	577	1933	F	R	12.5	N	15	Α
Isuituq         18/06/11         171         640         3035         F         R         44.0         Y         19         A           Isuituq         18/06/11         172         570         261         M         R         1.5         N         10         A           Isuituq         18/06/11         173         561         1914         F         R         6.0         Y         12         A           Isuituq         18/06/11         176         616         2466         F         R         0.5         Y         8         A           Isuituq         18/06/11         177         597         2267         U         R         98.5         Y         14         A           Isuituq         18/06/11         178         534         1634         M         R         0.5         N         11         A           Isuituq         18/06/11         161         609         2360         F         R         9.0         N         14         A           Isuituq         18/06/11         163         462         1286         F         1         3.5         N         10         A           Isuituq	 Isuituq	18/08/11	170	320	340	М	l	0.5	Y	7	A
Isuituq         18/06/11         172         570         2461         M         R         1.5         N         10         A           Isuituq         18/06/11         173         561         1914         F         R         6.0         Y         17         A           Isuituq         18/06/11         176         652         3211         M         R         8.0         Y         17         A           Isuituq         18/06/11         176         616         2466         F         R         12.0         Y         13         A           Isuituq         18/06/11         176         534         1634         M         R         0.5         N         11         A           Isuituq         18/06/11         178         534         1634         M         R         0.5         N         11         A           Isuituq         18/06/11         180         652         3492         F         R         120.0         N         20         A           Isuituq         18/06/11         182         464         1261         F         R         7.0         N         9         A           Isuituq	 lsuituq	18/08/11	171	640	3035	F	R	44.0	Y	19	Α
Isuituq         18/06/11         173         561         19/14         F         R         6.0         Y         12         A           Isuituq         18/06/11         174         652         3211         M         R         0.5         Y         8         A           Isuituq         18/06/11         176         616         2466         F         R         12.0         Y         13         A           Isuituq         18/06/11         177         597         2267         U         R         98.5         Y         14         A           Isuituq         18/06/11         179         644         3115         M         R         4.5         N         11         A           Isuituq         18/06/11         180         652         3462         F         R         120.0         N         20         A           Isuituq         18/06/11         181         6609         2360         F         R         120.0         N         14         A           Isuituq         18/06/11         182         442         1286         F         I         3.5         N         10.0         A           Isuitu	 Isuituq	18/08/11	172	570	2461	М	R	1.5	N	10	Α
Isuituq         18/08/11         174         652         3211         M         R         8.0         Y         17         A           Isuituq         18/08/11         175         440         082         M         R         0.5         Y         8         A           Isuituq         18/08/11         176         646         2466         F         R         12.0         Y         13         A           Isuituq         18/08/11         176         644         3115         M         R         0.5         N         11         A           Isuituq         18/08/11         180         652         3492         F         R         129.0         N         20         A           Isuituq         18/06/11         181         609         2360         F         R         9.0         N         14         A           Isuituq         18/06/11         183         482         1284         F         R         7.0         N         9         A           Isuituq         18/06/11         183         482         1284         F         R         10.0         N         16         A           Isuituq	Isuituq	18/08/11	173	561	1914	F	R	6.0	Y	12	Α
Isuituq         18/08/11         175         440         1082         M         R         0.5         Y         8         A           Isuituq         18/08/11         176         616         2466         F         R         12.0         Y         13         A           Isuituq         18/08/11         177         597         2267         U         R         98.5         Y         14         A           Isuituq         18/08/11         178         534         1634         M         R         0.5         N         11         A           Isuituq         18/08/11         180         652         3492         F         R         12.0         N         20         A           Isuituq         18/08/11         181         609         2360         F         R         9.0         N         14         A           Isuituq         18/08/11         182         484         1261         F         R         7.0         N         9         A           Isuituq         18/06/11         183         440         912         M         R         0.5         Y         7         A           Isuituq	Isuituq	18/08/11	174	652	3211	М	R	8.0	Y	17	Α
Isuituq         18/08/11         176         616         2466         F         R         12.0         Y         13         A           Isuituq         18/08/11         177         557         2267         U         R         98.5         Y         14         A           Isuituq         18/08/11         178         534         1634         M         R         0.5         N         11         A           Isuituq         18/08/11         180         652         3492         F         R         12.0         N         20         A           Isuituq         18/08/11         181         609         2360         F         R         9.0         N         14         A           Isuituq         18/08/11         182         484         1281         F         R         7.0         N         9         A           Isuituq         18/08/11         183         482         1288         F         I         3.5         N         10         A           Isuituq         18/08/11         186         627         2745         M         R         2.0         N         16         A           Isuituq	Isuituq	18/08/11	175	440	1082	М	R	0.5	Y	8	Α
Isuituq         18/08/11         177         597         2267         U         R         98.5         Y         14         A           Isuituq         18/08/11         178         534         1634         M         R         0.5         N         111         A           Isuituq         18/08/11         180         652         3492         F         R         120         N         20         A           Isuituq         18/08/11         181         609         2360         F         R         9.0         N         14         A           Isuituq         18/08/11         182         484         1261         F         R         9.0         N         14         A           Isuituq         18/08/11         183         482         1288         F         I         3.5         N         10         A           Isuituq         18/08/11         185         644         1803         F         R         13.0         Y         18         A           Isuituq         18/08/11         187         604         1803         F         R         13.0         Y         18         A           Isuituq <td> Isuituq</td> <td>18/08/11</td> <td>176</td> <td>616</td> <td>2466</td> <td>F</td> <td>R</td> <td>12.0</td> <td>Y</td> <td>13</td> <td>Α</td>	 Isuituq	18/08/11	176	616	2466	F	R	12.0	Y	13	Α
	 Isuituq	18/08/11	177	597	2267	U	R	98.5	Y	14	Α
	 Isuituq	18/08/11	178	534	1634	М	R	0.5	N	11	Α
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	 Isuituq	18/08/11	179	644	3115	М	R	4.5	N	11	A
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	180	652	3492	F	R	129.0	N	20	Α
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	181	609	2360	F	R	9.0	N	14	Α
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	 Isuituq	18/08/11	182	484	1261	F	R	7.0	N	9	Α
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Isuituq	18/08/11	183	482	1288	F	I	3.5	N	10	Α
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	184	627	2745	М	R	2.0	N	12	Α
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	185	440	912	М	R	0.5	Y	7	Α
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Isuituq	18/08/11	186	591	1849	F	R	19.0	Ν	16	А
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Isuituq	18/08/11	187	604	1803	F	R	13.0	Y	18	А
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Isuituq	18/08/11	188	560	1561	F	R	37.5	Ν	8	Α
Isuituq18/08/111905292083FM94.0N14AIsuituq18/08/111914841241FM30.5N9AIsuituq18/08/111924931306MR1.5Y10AIsuituq18/08/11193377546MI<.5	 Isuituq	18/08/11	189	448	906	М	R	1.5	Y	15	A
Isuituq         18/08/11         191         484         1241         F         M         30.5         N         9         A           Isuituq         18/08/11         192         493         1306         M         R         1.5         Y         10         A           Isuituq         18/08/11         193         377         546         M         I         <.5	Isuituq	18/08/11	190	529	2083	F	М	94.0	N	14	A
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Isuituq	18/08/11	191	484	1241	F	М	30.5	N	9	A
Isuituq18/08/11193377546MI<.5N7AIsuituq18/08/111944891267MI0.5Y13AIsuituq18/08/111954701130MR0.5Y7AIsuituq18/08/11196422889FI3.0Y8AIsuituq18/08/11197464917MR0.5N8AIsuituq18/08/111985131539FR15.0Y9AIsuituq18/08/111994691339FR7.0Y10AIsuituq18/08/11200393680MI0.5N7AIsuituq18/08/112016773815FR65.0Y15AKipisa24/08/112035111421MR2.5Y10AKipisa24/08/112044631029MR0.5N7AKipisa24/08/112055422018MR1.5N7AKipisa24/08/112065802454MR4.5N114AKipisa24/08/112075551946FM153.5N15AKipisa24/08/11206580<	Isuituq	18/08/11	192	493	1306	М	R	1.5	Y	10	A
Isuituq         18/08/11         194         489         1267         M         I         0.5         Y         13         A           Isuituq         18/08/11         195         470         1130         M         R         0.5         Y         7         A           Isuituq         18/08/11         196         422         889         F         I         3.0         Y         8         A           Isuituq         18/08/11         197         464         917         M         R         0.5         N         8         A           Isuituq         18/08/11         198         513         1539         F         R         15.0         Y         9         A           Isuituq         18/08/11         199         469         1339         F         R         7.0         Y         10         A           Isuituq         18/08/11         200         393         680         M         I         0.5         N         7         A           Isuituq         18/08/11         201         677         3815         F         R         65.0         Y         15         A           Kipisa         <	Isuituq	18/08/11	193	377	546	М	I	<.5	N	7	A
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Isuituq	18/08/11	194	489	1267	М	I	0.5	Y	13	Α
Isuituq         18/08/11         196         422         889         F         I         3.0         Y         8         A           Isuituq         18/08/11         197         464         917         M         R         0.5         N         8         A           Isuituq         18/08/11         198         513         1539         F         R         15.0         Y         9         A           Isuituq         18/08/11         199         469         1339         F         R         7.0         Y         10         A           Isuituq         18/08/11         200         393         680         M         I         0.5         N         7         A           Isuituq         18/08/11         201         677         3815         F         R         65.0         Y         15         A           Kipisa         24/08/11         202         421         915         M         R         0.5         N         7         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa	 Isuituq	18/08/11	195	470	1130	М	R	0.5	Y	7	Α
Isuituq         18/08/11         197         464         917         M         R         0.5         N         8         A           Isuituq         18/08/11         198         513         1539         F         R         15.0         Y         9         A           Isuituq         18/08/11         199         469         1339         F         R         7.0         Y         10         A           Isuituq         18/08/11         200         393         680         M         I         0.5         N         7         A           Isuituq         18/08/11         201         677         3815         F         R         65.0         Y         15         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa <t< td=""><td>Isuituq</td><td>18/08/11</td><td>196</td><td>422</td><td>889</td><td>F</td><td>I</td><td>3.0</td><td>Y</td><td>8</td><td>A</td></t<>	Isuituq	18/08/11	196	422	889	F	I	3.0	Y	8	A
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	197	464	917	М	R	0.5	Ν	8	А
Isuituq         18/08/11         199         469         1339         F         R         7.0         Y         10         A           Isuituq         18/08/11         200         393         680         M         I         0.5         N         7         A           Isuituq         18/08/11         201         677         3815         F         R         65.0         Y         15         A           Kipisa         24/08/11         202         421         915         M         R         0.5         N         7         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         204         463         1029         M         R         0.5         N         133         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa <td< td=""><td> Isuituq</td><td>18/08/11</td><td>198</td><td>513</td><td>1539</td><td>F</td><td>R</td><td>15.0</td><td>Y</td><td>9</td><td>Α</td></td<>	 Isuituq	18/08/11	198	513	1539	F	R	15.0	Y	9	Α
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	 Isuituq	18/08/11	199	469	1339	F	R	7.0	Y	10	Α
Isuituq         18/08/11         201         677         3815         F         R         65.0         Y         15         A           Kipisa         24/08/11         202         421         915         M         R         0.5         N         7         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         204         463         1029         M         R         0.5         N         13         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         15         A           Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa <t< td=""><td> Isuituq</td><td>18/08/11</td><td>200</td><td>393</td><td>680</td><td>М</td><td>l</td><td>0.5</td><td>N</td><td>7</td><td>Α</td></t<>	 Isuituq	18/08/11	200	393	680	М	l	0.5	N	7	Α
Kipisa         24/08/11         202         421         915         M         R         0.5         N         7         A           Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         204         463         1029         M         R         0.5         N         13         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         15         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         11         A           Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa <t< td=""><td> Isuituq</td><td>18/08/11</td><td>201</td><td>677</td><td>3815</td><td>F</td><td>R</td><td>65.0</td><td>Y</td><td>15</td><td>Α</td></t<>	 Isuituq	18/08/11	201	677	3815	F	R	65.0	Y	15	Α
Kipisa         24/08/11         203         511         1421         M         R         2.5         Y         10         A           Kipisa         24/08/11         204         463         1029         M         R         0.5         N         13         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         15         A           Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa         24/08/11         209         523         1978         F         R         9.0         Y         9         A           Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa <td< td=""><td> Kipisa</td><td>24/08/11</td><td>202</td><td>421</td><td>915</td><td>М</td><td>R</td><td>0.5</td><td>Ν</td><td>7</td><td>A</td></td<>	 Kipisa	24/08/11	202	421	915	М	R	0.5	Ν	7	A
Kipisa         24/08/11         204         463         1029         M         R         0.5         N         13         A           Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         15         A           Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa         24/08/11         209         523         1978         F         R         9.0         Y         9         A           Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa <td< td=""><td> Kipisa</td><td>24/08/11</td><td>203</td><td>511</td><td>1421</td><td>М</td><td>R</td><td>2.5</td><td>Y</td><td>10</td><td>A</td></td<>	 Kipisa	24/08/11	203	511	1421	М	R	2.5	Y	10	A
Kipisa         24/08/11         205         542         2018         M         R         1.5         N         7         A           Kipisa         24/08/11         206         580         2454         M         R         4.5         N         14         A           Kipisa         24/08/11         207         555         1946         F         M         153.5         N         15         A           Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa         24/08/11         209         523         1978         F         R         9.0         Y         9         A           Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa         24/08/11         212         665         3446         M         R         3.0         N         12         A	 Kipisa	24/08/11	204	463	1029	М	R	0.5	Ν	13	A
Kipisa24/08/112065802454MR4.5N14AKipisa24/08/112075551946FM153.5N15AKipisa24/08/112085161279FR8.5N11AKipisa24/08/112095231978FR9.0Y9AKipisa24/08/112104761401MR1.5N12AKipisa24/08/112117194019MR8.0N15AKipisa24/08/112126653446MR3.0N12A	 Kipisa	24/08/11	205	542	2018	М	R	1.5	Ν	7	A
Kipisa24/08/112075551946FM153.5N15AKipisa24/08/112085161279FR8.5N11AKipisa24/08/112095231978FR9.0Y9AKipisa24/08/112104761401MR1.5N12AKipisa24/08/112117194019MR8.0N15AKipisa24/08/112126653446MR3.0N12A	 Kipisa	24/08/11	206	580	2454	М	R	4.5	Ν	14	A
Kipisa         24/08/11         208         516         1279         F         R         8.5         N         11         A           Kipisa         24/08/11         209         523         1978         F         R         9.0         Y         9         A           Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa         24/08/11         212         665         3446         M         R         3.0         N         12         A	Kipisa	24/08/11	207	555	1946	F	М	153.5	N	15	Α
Kipisa         24/08/11         209         523         1978         F         R         9.0         Y         9         A           Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa         24/08/11         212         665         3446         M         R         3.0         N         12         A	 Kipisa	24/08/11	208	516	1279	F	R	8.5	N	11	Α
Kipisa         24/08/11         210         476         1401         M         R         1.5         N         12         A           Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa         24/08/11         212         665         3446         M         R         3.0         N         12         A	 Kipisa	24/08/11	209	523	1978	F	R	9.0	Y	9	A
Kipisa         24/08/11         211         719         4019         M         R         8.0         N         15         A           Kipisa         24/08/11         212         665         3446         M         R         3.0         N         12         A	 Kipisa	24/08/11	210	476	1401	М	R	1.5	N	12	Α
Kipisa 24/08/11 212 665 3446 M R 3.0 N 12 A	 Kipisa	24/08/11	211	719	4019	М	R	8.0	Ν	15	A
	 Kipisa	24/08/11	212	665	3446	М	R	3.0	Ν	12	A

 Kipisa	24/08/11	213	468	1257	F	R	4.5	N	10	A
 Kipisa	24/08/11	214	439	995	F	Μ	88.0	Y	12	A
 Kipisa	24/08/11	215	462	1142	F		5.5	Ν	9	A
Kipisa	24/08/11	216	681	3244	Μ	R	7.0	Ν	14	A
Kipisa	24/08/11	217	518	1755	Μ	R	1.5	Ν	9	A
Kipisa	24/08/11	218	552	2256	Μ	R	21.5	Ν	10	A
Kipisa	24/08/11	219	472	1169	Μ	R	10.0	Y	9	A
 Kipisa	24/08/11	220	612	2999	F	R	1.0	Ν	12	A
 Kipisa	24/08/11	221	622	2802	F	R	1.0	Ν	14	A
 Kipisa	24/08/11	222	495	1374	Μ	1	8.0	Ν	8	A
 Kipisa	24/08/11	223	523	1831	Μ	R	1.0	N	8	A
 Kipisa	24/08/11	224	507	1754	F	R	2.0	Ν	10	A
 Kipisa	24/08/11	225	475	1319	Μ	R	13.0	Ν	9	A
 Kipisa	24/08/11	226	406	768	F	R	1.0	Ν	9	A
 Kipisa	24/08/11	227	568	1677	F	R	2.0	Y	12	A
 Kipisa	24/08/11	228	427	909	Μ	R	4.5	N	7	A
 Kipisa	24/08/11	229	566	558	F	<u> </u>	2.0	N	8	A
 Kipisa	24/08/11	230	670	4379	Μ	R	0.5	Y	11	A
 Kipisa	24/08/11	231	514	1771	Μ	R	0.5	Y	10	A
 Kipisa	24/08/11	232	404	776	M	R	0.5	N	8	A
 Kipisa	24/08/11	233	368	652	F		<0.5	N	8	A
 Kipisa	24/08/11	234	409	809	M	R	1.0	N	10	A
 Kipisa	24/08/11	235	298	308	M		4.5	N	7	A
 Kipisa	24/08/11	236	328	424	F		7.5	N	6	A
 Kipisa	24/08/11	237	413	893	F		5.5	Y	9	A
 Kipisa	24/08/11	238	693	4118	M	R	7.5	Y	19	A
 Kipisa	24/08/11	239	500	1561	F	R	3.5	N	11	A
 Kipisa	24/08/11	240	566	1812	F	R	1.0	N	9	A
 Kipisa	24/08/11	241	441	984	F	R	1.0	N	8	A
 Kipisa	24/08/11	242	449	950	M	R	6.0	N	8	A
 Kipisa	24/08/11	243	519	1475	M	R	0.5	Y	10	A
 Kipisa	24/08/11	244	726	5160	M	R	3.5	Y	12	A
 Kipisa	24/08/11	245	401	787	M	R	14.5	N	8	A
 Kipisa	24/08/11	246	643	3876	M	R	5.0	N	11	A
 Kipisa	24/08/11	247	532	1963	F	R	0.5	N	11	A
 Kipisa	24/08/11	248	740	3058	M	R	10.0	Y	14	A
 Kipisa	24/08/11	249	511	1388	M	R	2.5	Y	9	A
 Kipisa	24/08/11	250	436	1247	F	R	0.5	Y	14	A
 Kipisa	24/08/11	251	469	1110	F	R	<.5	N	9	A
 Kipisa	24/08/11	252	437	1016	M	R	6.0	N	9	A
 Kipisa	24/08/11	253	326	425	Μ	<u> </u>	2.0	N	7	A
 Kipisa	24/08/11	254	470	1261	F	R	1.5	N		A
 Kipisa	24/08/11	255	449	1152	F		<.5	N	9	A
 Kipisa	24/08/11	256	362	485	F	1	<.5	Y	9	A

 Kipisa	24/08/11	257	360	505	М	I	<.5	Y	6	A
Kipisa	24/08/11	258	283	287	F	I	<.5	Y	6	А
 Kipisa	24/08/11	259	290	252	F	I	<.5	Ν	6	Α
 Kipisa	24/08/11	260	326	420	F	I	<.5	Y	6	Α
 Kipisa	24/08/11	261	300	339	М	l	<.5	Ν	6	Α
 Kipisa	24/08/11	262	281	265	Μ	I	<.5	N	5	Α
 Kipisa	24/08/11	263	445	949	F	I	3.5	Y	9	Α
 Kipisa	24/08/11	264	402	779	Μ	I	0.5	N	9	Α
 Kipisa	24/08/11	265	367	562	M	I	0.5	Y	6	Α
 Kipisa	24/08/11	266	366	391	M	1	<.5	N	6	Α
 Kipisa	24/08/11	267	572	2322	F	R	22.0	Y	11	Α
 Kipisa	24/08/11	268	538	2131	M	R	1.0	N	11	Α
 Kipisa	24/08/11	269	402	650	F	l	4.5	N	10	Α
 Kipisa	24/08/11	270	375	642	M	l	1.0	N	7	Α
 Kipisa	24/08/11	271	466	1190	M	l	0.5	N	8	Α
 Kipisa	24/08/11	272	412	820	F	I	3.0	N	9	Α
 Kipisa	24/08/11	273	404	755	Μ	l	0.5	N	8	Α
 Kipisa	24/08/11	274	395	718	F	I	2.0	N	9	A
 Kipisa	24/08/11	275	417	870	F	I	2.5	N	7	A
 Kipisa	24/08/11	276	305	379	M		0.5	Y	6	Α
 Kipisa	24/08/11	277	359	451	F	l	2.0	Y	8	Α
 Kipisa	24/08/11	278	662	4175	F	R	28.5	Y	14	Α
 Kipisa	24/08/11	279	595	3219	Μ	R	3.0	Y	11	Α
 Kipisa	24/08/11	280	416	790	F	I	4.5	Y	8	Α
 Kipisa	24/08/11	281	418	579	F		4.5	Y	9	Α
 Kipisa	24/08/11	282	371	650	F		1.0	Y	7	A
 Kipisa	24/08/11	283	287	289	M	I	0.5	N	4	Α
 Kipisa	24/08/11	284	349	421	Μ	R	1.0	Y	6	Α
 Kipisa	24/08/11	285	324	392	Μ	l	0.5	N	7	Α
 Kipisa	24/08/11	286	281	266	F	I	0.5	N	6	Α
 Kipisa	24/08/11	287	386	631	M	I	<.5	N	7	Α
 Kipisa	24/08/11	288	379	642	F	I	1.5	N	6	Α
 Kipisa	24/08/11	289	295	324	M	i	<.5	Y	8	A
 Kipisa	24/08/11	290	325	462	F	I	0.5	Ň	5	A
 Kipisa	24/08/11	291	303	337	M	I	0.5	N	5	Α
 Kipisa	24/08/11	292	283	259	M		<.5	N	7	A
 Kipisa	25/08/11	293	434	932	M		0.5	N	8	A
 Kipisa	25/08/11	294	405	707	M		0.5	N	8	A
 Kipisa	25/08/11	295	282	340	F	i	0.5	N	9	A
 Kipisa	25/08/11	296	442	1091	M		0.5	Y	8	A
 Kipisa	25/08/11	297	451	1062	M	I	0.5	Ý	8	Α
 Kinisa	25/08/11	298	420	847	F		2 5	N	8	Α
 Kipisa	25/08/11	299	424	836	M		0.5	N	7	Α
 Kinisa	25/08/11	300	271	232	F		1.0	N	6	Δ
 inpida	20,00/11	000	<u> </u>	202	1	I	1.0	: 4	~	/ <b>`</b>

 Kipisa	25/08/11	301	280		Μ	l	<.5	U	5	A
 Kipisa	25/08/11	302	756	4123	Μ	R	6.5	Y	16	A
 Kipisa	25/08/11	303	666	3889	Μ	R	8.5	Y	12	A
 Kipisa	25/08/11	304	340	457	F	I	0.5	Y	7	Α
Kipisa	25/08/11	305	304	310	F	I	0.5	N	4	A
Kipisa	25/08/11	306	357	557	F	I	1.0	Ν	6	A
Kipisa	25/08/11	307	340	439	F	R	1.5	Ν	9	Α
 Kipisa	25/08/11	308	294	280	Μ	I	<.5	Y	6	A
 Kipisa	25/08/11	309	288	259	F	I	0.5	N	5	A
 Kipisa	25/08/11	310	329	420	Μ	I	0.5	Ν	7	A
 Kipisa	25/08/11	311	310	326	Μ	I	<.5	Ν	6	A
 Kipisa	25/08/11	312	275		Μ	I	<.5	Y	5	A
 Kipisa	25/08/11	313	383	673	F	I	2.5	Y	8	A
 Kipisa	25/08/11	314	347	404	Μ	I	0.5	N	6	A
 Kipisa	25/08/11	315	306		Μ	I	<.5	N	7	A
 Kipisa	25/08/11	316	278		U	U	U	U	5	A
 Kipisa	25/08/11	317	286		F	1	0.5	Y	4	A
 Kipisa	25/08/11	318	309		Μ	1	0.5	Y	7	A
 Kipisa	25/08/11	319	300	285	Μ	1	<.5	N	7	A
 Kipisa	25/08/11	320	305		Μ	I	0.5	N	7	A
 Kipisa	25/08/11	321	323		F	I	0.5	N	4	A
 Kipisa	25/08/11	322	294		Μ	l	0.5	Y	6	A
 Kipisa	25/08/11	323	275		F	1	0.5	N	6	A
 Kipisa	25/08/11	324	616	1645	F	R	31.0	N	15	A
 Kipisa	25/08/11	325	405	723	F	1	2.0	N	6	A
 Kipisa	25/08/11	326	231	137	М	1	<.5	Ν	5	A
 Kipisa	25/08/11	327	279	257	F	l	0.5	N	5	A
 Kipisa	25/08/11	328	182	56	Μ	I	<.5	N	5	A
 Kipisa	25/08/11	329	273	219	Μ	I	<.5	N	6	A
 Kipisa	25/08/11	330	266	230	F	I	0.5	Y	5	A
 Kipisa	25/08/11	331	350	499	Μ	l	0.5	N	6	A
 Kipisa	25/08/11	332	331	328	F	I	1.5	Y	8	A
 Kipisa	25/08/11	333	307		Μ	1	<.5	N	5	A
 Kipisa	25/08/11	334	181	60	F	1	<.5	N	3	A
 Kipisa	25/08/11	335	190	70	F	1	<.5	N	5	A
 Kipisa	25/08/11	336	315	330	Μ	1	0.5	N	8	A
 Kipisa	25/08/11	337	250	160	Μ	1	<.5	N	5	A
 Kipisa	25/08/11	338	267	196	F	1	0.5	Y	5	A
 Kipisa	25/08/11	339	283	244	F	1	0.5	Y	4	A
 Kipisa	25/08/11	340	284		Μ	1	0.5	N	6	A
 Kipisa	25/08/11	341			Μ			U		A
 Kipisa	25/08/11	342			U	U		Ū		A
 Kipisa	25/08/11	343	601	2566	F	R	15.5	Ŷ	14	A
 Kipisa	25/08/11	344	456	1234	M	R	1.0	N	7	A
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 Kipisa	25/08/11	345	380	737	Μ	R	1.0	Ν	7	A
 Kipisa	25/08/11	346	426	889	Μ	R	1.5	Ν	7	A
Kipisa	25/08/11	347	280	269	Μ		0.5	Ν	4	A
 Kipisa	25/08/11	348	286	263	Μ	l	<.5	N	5	A
 Kipisa	25/08/11	349	298	285	Μ	R	0.5	N	5	A
 Kipisa	25/08/11	350	580	2370	Μ	R	2.5	N	12	A
 Kipisa	25/08/11	351	403	776	Μ	R	0.5	N	9	A
 Kipisa	25/08/11	352	665	4267	Μ	R	4.0	Y	12	A
 Kipisa	25/08/11	353	236	141	Μ	l	<.5	N	5	A
 Kipisa	25/08/11	354	503	1844	F	R	9.5	N	9	A
 Kipisa	25/08/11	355	614	2639	F	R	17.0	N	16	A
 Kipisa	25/08/11	356	646	1945	F	R	23.5	N	18	A
 Kipisa	25/08/11	357	597	2720	Μ	R	2.5	N	14	A
 Kipisa	25/08/11	358	677	3310	Μ	R	5.0	N	15	A
 Kipisa	25/08/11	359	374	695	Μ	R	0.5	N	7	A
 Kipisa	25/08/11	360	452	1230	Μ	R	1.0	N	8	A
 Kipisa	25/08/11	361	519	1790	F	R	10.0	N	10	A
 Kipisa	25/08/11	362	448	1231	F	R	4.5	Y	9	A
 Kipisa	25/08/11	363	365	570	Μ	I	0.5	N	8	A
 Kipisa	25/08/11	364	450	1018	Μ	R	0.5	N	10	A
 Kipisa	25/08/11	365	414	848	F	l	1.5	N	7	A
 Kipisa	25/08/11	366	479	1513	F	R	6.0	Y	7	A
 Kipisa	25/08/11	367	705	3858	Μ	R	9.5	N	18	A
 Kipisa	25/08/11	368	547	2189	F	R	7.5	Y	8	A
 Kipisa	25/08/11	369	624	2675	Μ	R	5.5	Y	16	A
 Kipisa	25/08/11	370	469	1313	Μ	R	1.0	N	9	A
 Kipisa	25/08/11	371	592	2242	Μ	М	89.0	Y	20	A
 Kipisa	25/08/11	372	503	2085	Μ	R	3.5	Y	11 .	A
 Kipisa	25/08/11	373	542	2207	Μ	R	1.5	N	10	A
 Kipisa	25/08/11	374	321	386	F	l	1.0	N	7	A
 Kipisa	25/08/11	375	565	2001	F	R	13.0	N	12	A
 Kipisa	25/08/11	376	307	325	F	l	0.5	N	6	A
 Kipisa	25/08/11	377	374	618	F	I	2.0	N	8	A
 Kipisa	25/08/11	378	302	310	F		<.5	N	6	A
 Kipisa	25/08/11	379	270	201	Μ	l	<.5	N	6	A
 Kipisa	25/08/11	380	501	1526	F	R	8.0	N	10	A
 Kipisa	25/08/11	381	474	1172	F	R	7.0	Y	10	A
 Kipisa	25/08/11	382	370	595	Μ	I	0.5	N	8	A
 Kipisa	25/08/11	383	401	679	Μ	l	0.5	Y	10	A
 Kipisa	25/08/11	384	364	543	Μ	1	0.5	N	7	A
 Kipisa	25/08/11	385	337	427	F	1	1.0	N	6	A
 Kipisa	25/08/11	386	379	734	F	1	3.0	Y	9	A
 Kipisa	25/08/11	387	309	349	Μ	1	<.5	Y	5	A
 Kipisa	25/08/11	388	356	371	F	1	1.0	Y	9	A
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 Kipisa	25/08/11	389	350	451	F		0.1	Y	6	Α
Kipisa	25/08/11	390	327	373	М	I	0.1	Ν	9	A
 Kipisa		391	291	307	F		0.5	Y	5	Α
 Kipisa	25/08/11	392	315	361	М		<.5	Ν	5	Α
 Kipisa	25/08/11	393	303	329	F	l	1.5	Ν	7	Α
 Kipisa	25/08/11	394	310	312	F	l	1.0	Ν	8	А
 Kipisa	25/08/11	395	297	331	М	l	<.5	Y	5	А
 Kipisa	25/08/11	396	298	320	М	l	<.5	Y	7	А
Kipisa	25/08/11	397	422	773	М	l	0.5	Y	8	Α
Kipisa	25/08/11	398	524	1889	F	R	10.0	Y	10	Α
Qasigiyat	05/09/11	801	494	1275	М	М	33.5	Ν		Α
Qasigiyat	05/09/11	802	527	1775	F	М	207.0	Ν		Α
Qasigiyat	05/09/11	803	759	5350	М	М	270.5	Ν		Α
 Qasigiyat	05/09/11	804	625	2675	М	М	43.0	Ν		Α
 Qasigiyat	05/09/11	805	652	3500	М	М	90.0	Ν		А
Qasigiyat	05/09/11	806	758	6100	М	RR	129.5	Ν	-	A
 Qasigiyat	05/09/11	807	726	5000	М	RR	157.5	Ν		A
 Qasigiyat	06/09/11	808	169	49	М	U	0.5	Ν		U
 Qasigiyat	06/09/11	809	222	90	М		0.5	Ν		I
 Qasigiyat	06/09/11	810	201	68	F	l	0.5	N		I
 Qasigiyat	06/09/11	811	163	38	U	l	<0.5	Ν		I
 Qasigiyat	06/09/11	812	177	48	Μ	l	<0.5	Ν		I
 Qasigiyat	06/09/11	813	236	122	Μ	U	0.5	Ν		U
 Qasigiyat	06/09/11	814	160	38	М	М	2.5	Ν		R
 Qasigiyat	06/09/11	815	180	54	U		<0.5	Ν		I
 Qasigiyat	06/09/11	816	181	49	Μ	l	<0.5	N		I
 Qasigiyat	06/09/11	817	196	66	Μ	l	<0.5	N		l
 Qasigiyat	06/09/11	818	189	57	U	l	<0.5	Ν		I
 Qasigiyat	06/09/11	819	186	64	Μ	l	<0.5	N		I
 Qasigiyat	06/09/11	820	183	56	Μ	l	<0.5	N		I
 Qasigiyat	06/09/11	821	179	55	Μ	М	3.5	N		R
 Qasigiyat	06/09/11	822	123	16	U	l	<0.5	N		l
 Qasigiyat	06/09/11	823	176	53	F		<0.5	N		I
 Qasigiyat	06/09/11	824	148	27	U		<0.5	N		I
 Qasigiyat	06/09/11	825	150	26	U		<0.5	N		I
 Qasigiyat	06/09/11	826	173	54	F		<0.5	N		I
 Qasigiyat	06/09/11	827	207	74	F		<0.5	N		I
 Qasigiyat	06/09/11	828	513	1550	Μ	M	51.5	N		Α
 Qasigiyat	06/09/11	829	515	1600	F	R	8.5	N		Α
 Qasigiyat	06/09/11	830	642	1900	М	R	4.0	N		Α
 Qasigiyat	06/09/11	831	604	2950	Μ	R	2.5	N		Α
 Qasigiyat	06/09/11	832	584	2550	F	M	325.0	N		Α
 Qasigiyat	06/09/11	833	661	2050	Μ	R	6.0	N		Α
 Qasigiyat	06/09/11	834	539	2000	F	M	204.0	N		Α

 Qasigiyat	06/09/11	835	618	2800	М	М	126.0	N	A
 Qasigiyat	06/09/11	836	573	2300	F	М	327.0	N	A
 Qasigiyat	06/09/11	837	402	675	М	R	1.0	N	A
Qasigiyat	06/09/11	838	575	2300	F	М	274.0	Ν	A
Qasigiyat	06/09/11	839	615	2800	F	М	381.0	Ν	Â
Qasigiyat	06/09/11	840	595	2450	F	М	281.5	Ν	Â
Qasigiyat	06/09/11	841	561	2200	F	М	268.0	Ν	Â
 Qasigiyat	07/09/11	842	342	423	F	l	2.0	Ν	1
 Qasigiyat	07/09/11	843	646	3125	М	RR	97.0	N	Â
 Qasigiyat	07/09/11	844	168	38	Μ	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	845	201	73	F	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	846	146	30	M	M	2.0	N	R
 Qasigiyat	07/09/11	847	145	27	М	Μ	1.5	Ν	R
 Qasigiyat	07/09/11	848	192	59	М	l	<0.5	Ν	I
 Qasigiyat	07/09/11	849	178	49	М	<b>I</b>	<0.5	N	I
 Qasigiyat	07/09/11	850	216	99	F	<b>I</b>	<0.5	N	I
 Qasigiyat	07/09/11	851	181	58	F	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	852	180	51	Μ	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	853	234	128	M	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	854	139	25	F	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	855	237	121	М	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	856	175	53	М	M	3.0	N	R
 Qasigiyat	07/09/11	857	226	106	М	M	6.0	N	R
 Qasigiyat	07/09/11	858	257	173	M	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	859	111	13	U	<u> </u>	<0.5	Y	I
 Qasigiyat	07/09/11	860	638	3000	M	R	6.0	N	Α
 Qasigiyat	07/09/11	861	510	1450	Μ	R	1.5	N	Α
 Qasigiyat	07/09/11	862	748	5325	M	M	161.5	N	Α
 Qasigiyat	07/09/11	863	631	2850	М	M	120.0	N	Α
 Qasigiyat	07/09/11	864	724	4750	M	RR	193.5	N	Α
 Qasigiyat	07/09/11	865	674	3475	М	M	125.5	N	Α
 Qasigiyat	07/09/11	866	657	3550	M	RR	104.5	N	Α
 Qasigiyat	07/09/11	867	532	1850	F	M	164.5	N	Α
 Qasigiyat	07/09/11	868	511	1450	F	M	178.5	N	Α
 Qasigiyat	07/09/11	869	663	3250	M	RR	70.0	N	Α
 Qasigiyat	07/09/11	870	663	4000	M	M	158.5	N	Α
 Qasigiyat	07/09/11	871	185	58	М	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	872	239	112	М	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	873	178	45	F	<u> </u>	<0.5	N	I
 Qasigiyat	07/09/11	874	175	47	F	<b>I</b>	<0.5	N	I
 Qasigiyat	07/09/11	875	176	47	F		<0.5	Y	<u> </u>
 Qasigiyat	07/09/11	876	184	53	М	Μ	3.0	Y	R
 Qasigiyat	07/09/11	877	687	3400	М	M	145.5	N	Α
 Qasigiyat	07/09/11	878	657	3875	M	Μ	120.0	N	Α

 Qasigiyat	07/09/11	879	417	825	F		4.0	N	A
 Qasigiyat	07/09/11	880	423	825	М	l	<0.5	N	A
 Qasigiyat	07/09/11	881	207	83	М	l	<0.5	N	1
 Qasigiyat	07/09/11	882	209	84	F	l	<0.5	N	I
 Qasigiyat	07/09/11	883	178	47	М	R?	0.5	N	R?
 Qasigiyat	07/09/11	884	177	48	М	l	<0.5	N	I
 Qasigiyat	07/09/11	885	185	58	F	l	<0.5	N	I
 Qasigiyat	08/09/11	886	181	47	M	R	<0.5	N	R
 Qasigiyat	08/09/11	887	201	70	М	М	5.0	N	R
 Qasigiyat	08/09/11	888	189	64	M	R?	<0.5	N	R?
 Qasigiyat	08/09/11	889	188	60	M	l	<0.5	N	1
 Qasigiyat	08/09/11	890	204	72	F	l	<0.5	N	1
 Qasigiyat	08/09/11	891	182	54	M	l	<0.5	N	1
 Qasigiyat	08/09/11	892	233	130	F	l	<0.5	N	I
 Qasigiyat	08/09/11	893	200	75	U	l	<0.5	N	I
 Qasigiyat	08/09/11	894	174	47	М	l	<0.5	N	R?
 Qasigiyat	08/09/11	895	187	66	M	l	<0.5	N	R?
 Qasigiyat	08/09/11	896	194	62	М	l	<0.5	N	R?
 Qasigiyat	08/09/11	897	225	112	М	l	<0.5	N	1
 Qasigiyat	08/09/11	898	225	114	М	l	<0.5	N	<u> </u>
 Qasigiyat	08/09/11	899	228	105	F	<u> </u>	<0.5	N	I
 Qasigiyat	08/09/11	900	191	61	M	l	<0.5	Y	l
 Qasigiyat	08/09/11	901	151	31	F	l	<0.5	N	1
 Qasigiyat	08/09/11	902	118	15	F	l	<0.5	Y	1
 Qasigiyat	08/09/11	903	541	1700	М	R	1.5	N	Α
 Qasigiyat	08/09/11	904	370	517	F	l	1.0	N	Α
 Qasiqiyat	08/09/11	905	542	1975	F	Μ	220.5	N	Α
 Qasigiyat	09/09/11	906	192	61	M	l	<0.5	N	I
 Qasigiyat	09/09/11	907	177	55	M	l	<0.5	N	I
 Qasiqiyat	09/09/11	908	193	66	Μ	Μ	4.0	N	R
 Qasiqiyat	09/09/11	909	182	52	Μ	l	<0.5	N	I
 Qasiqiyat	09/09/11	910	149	23	F		<0.5	N	I
 Qasigivat	09/09/11	911	266	162	Μ		<0.5	N	I
 Qasiqiyat	09/09/11	912	334	368	F		0.5	N	
 Qasiqiyat	09/09/11	913	146	26	F		<0.5	N	
 Qasiqiyat	09/09/11	914	107	11	Μ		<0.5	N	
 Qasiqiyat	09/09/11	915	199	67	U	l	<0.5	N	
 Qasigivat	09/09/11	916	159	31	M		<0.5	N	I
 Qasigivat	09/09/11	917	190	62	F		<0.5	N	
 Qasigivat	09/09/11	918	176	53	Μ	Μ	2.5	N	R
 Qasiqiyat	09/09/11	919	303	271	M		<0.5	N	
 Qasigivat	09/09/11	920	130	21	M	M	1.5	N	R
 Qasiqiyat	09/09/11	921	164	46	M	M	2.5	Y	R
 Qasigiyat	09/09/11	922	187	56	F	I	<0.5	N	<u> </u>
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Qasigiyat	09/09/11	923	180	49	M		<0.5	<u>N</u>	<u> </u>
Qasigiyat	09/09/11	924	164	34	M	R	<0.5	Y	R
Qasigiyat	09/09/11	925	184	58	M	<u> </u>	<0.5	N	I
Qasigiyat	09/09/11	926	195	69	M	<u> </u>	<0.5	N	I
Qasigiyat	09/09/11	927	143	23	Μ	<u> </u>	<0.5	N	I
Qasigiyat	09/09/11	928	83	5	U	U	<0.5	N	
Qasigiyat	09/09/11	929	114	13	Μ	I	<0.5	N	R?
Qasigiyat	09/09/11	930	107	11	М	Μ	0.5	Y	R
Qasigiyat	09/09/11	931	111	12	М	I	<0.5	Y	I
Qasigiyat	09/09/11	932	437	900	Μ	М	43.5	N	A
Iqalugaarjuit	14/09/11	601	183	55	F	I	<0.5	Ν	I
Iqalugaarjuit	14/09/11	602	190	66	М	R	<0.5	N	R
Iqalugaarjuit	14/09/11	603	147	26	М	l	<0.5	N	R
Iqalugaarjuit	14/09/11	604	123	16	М	l	<0.5	Y	R
Iqalugaarjuit	14/09/11	605	196	61	F	l	<0.5	N	I
Iqalugaarjuit	14/09/11	606	178	50	F	I	<0.5	N	1
Iqalugaarjuit	14/09/11	607	205	86	М	1	<0.5	N	1
Igalugaarjuit	14/09/11	608	245	135	F	l	<0.5	N	1
Igalugaarjuit	14/09/11	609	207	186	М	l	<0.5	N	<u> </u>
Iqalugaarjuit	14/09/11	610	175	51	М	l	<0.5	N	<u> </u>
Igalugaarjuit	14/09/11	611	641	3679	F	RR	652.0	N	Α
Iqalugaarjuit	14/09/11	612	704	4050	М	RR	131.0	N	Α
Iqalugaarjuit	14/09/11	613	631	2653	F	RR	531.5	N	Α
Iqalugaarjuit	14/09/11	614	669	3503	М	М	99.5	N	Α
Iqalugaarjuit	15/09/11	615	223	89	М	М	3.0	N	R
Iqalugaarjuit	15/09/11	616	268	194	М	l	<0.5	N	1
Iqalugaarjuit	15/09/11	617	177	45	F	I	<0.5	Y	R
Iqalugaarjuit	15/09/11	618	157	36	М	М	0.5	N	R
Iqalugaarjuit	15/09/11	619	184	55	М	l	<0.5	N	I
Iqalugaarjuit	15/09/11	620	272	199	F	l	0.5	N	I
Igalugaarjuit	15/09/11	621	534	1827	F	М	304.5	N	Α
Iqalugaarjuit	15/09/11	622	175	56	F	1	<0.5	N	1
Igalugaarjuit	15/09/11	623	232	114	М	l	<0.5	N	<u> </u>
Igalugaarjuit	15/09/11	624	187	55	М	l	<0.5	N	<u> </u>
Iqalugaarjuit	15/09/11	625	190	70	М	l	<0.5	N	
Iqalugaarjuit	15/09/11	626	189	61	F	l	<0.5	N	<u> </u>
Iqalugaarjuit	15/09/11	627	219	103	F	l	<0.5	N	<u> </u>
Iqalugaarjuit	15/09/11	628	206	85	М	l	<0.5	N	<u> </u>
Igalugaarjuit	15/09/11	629	178	49	F	l	<0.5	N	1
Igalugaarjuit	15/09/11	630	550	2134	F	RR	413.5	N	Α
Igalugaarjuit	15/09/11	631	554	2118	F	Μ	393.0	N	Α
Igalugaarjuit	15/09/11	632	411	654	Μ	R	0.5	Y	A?
Iqalugaarjuit	15/09/11	633	617	2628	М	RR	68.5	N	Α
Iqalugaarjuit	16/09/11	634	198	82	М	l	<0.5	N	I
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Iqalugari I16/09/1163514723FI-0.5NIqalugari I16/09/1163618757MI<0.5NIqalugari I16/09/1163719265MR1.5NIqalugari I16/09/116395231760FM310.0NIqalugari I16/09/11640290247MI0.5NIqalugari I16/09/116415411800FM291.5NIqalugari I16/09/11642242138FI1.0NIqalugari I16/09/1164317855MM1.5NIqalugari I16/09/1164419162MI-0.5NIqalugari I16/09/1164618057FI-0.5NIqalugari I16/09/1164620281MI-0.5NIqalugari I16/09/11649258192FI-0.5NIqalugari I16/09/1165120285FI-0.5NIqalugari I16/09/1165120285FI-0.5NIqalugari I16/09/1165120285FI-0.5NIqalugari I16/09/1165519066MI-0.5 <th></th>										
Iqalugarjuit       16/09/11       636       187       57       M       I $\circ O_5$ N         Iqalugarjuit       16/09/11       638       410       668       M       R       0.5       Y         Iqalugarjuit       16/09/11       639       523       1760       F       M       310.0       N         Iqalugarjuit       16/09/11       640       290       247       M       I       0.5       N         Iqalugarjuit       16/09/11       644       290       247       M       I       0.5       N         Iqalugarjuit       16/09/11       644       290       247       M       I       0.5       N         Iqalugarjuit       16/09/11       644       191       65       M       M       1.5       N         Iqalugarjuit       16/09/11       646       190       57       F       I $< < > 0.5$ N         Iqalugarjuit       16/09/11       646       198       72       M       I $< < > 0.5$ N         Iqalugarjuit       16/09/11       649       285       192       F       I $< < > 0.5$ N         Iqaluga	Iqalugaarjuit	16/09/11	635	147	23	F	I	<0.5	N	R
$\begin{tabular}{  c   c   c   c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	636	187	57	М	I	<0.5	N	I
$\begin{tabular}{  c   c   c   c   c  c  c  c  c  c  c  $	Iqalugaarjuit	16/09/11	637	192	65	М	RR	1.5	N	R
$\begin{tabular}{l l l l l l l l l l l l l l l l l l l $	Iqalugaarjuit	16/09/11	638	410	668	М	R	0.5	Y	A?
$\begin{tabular}{  c  c  c  c  c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	639	523	1760	F	М	310.0	N	Α
$\begin{tabular}{  c  c  c  c  c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	640	290	247	М	I	0.5	N	I
$\begin{tabular}{  c  c  c  c  c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	641	541	1800	F	М	291.5	Ν	A
$\begin{tabular}{  c  c  c  c  c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	642	242	138	F	I	1.0	Ν	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	643	178	55	М	М	1.5	N	R
$\begin{tabular}{  c  c  c  c  c  c  c  c  c  c  c  c  c$	Iqalugaarjuit	16/09/11	644	191	62	M	<u> </u>	<0.5	N	I
$\begin{tabular}{ l   l   l   l   l   l   l   l   l   l$	Iqalugaarjuit	16/09/11	645	180	57	F	<u> </u>	<0.5	N	I
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	646	202	81	M	<u> </u>	<0.5	N	I
$\begin{tabular}{ l  l  l  l  l  l  l  l  l  l  l  l  l $	Iqalugaarjuit	16/09/11	647	198	74	M	<u> </u>	<0.5	N	I
$\begin{tabular}{ l  l  l  l  l  l  l  l  l  l  l  l  l $	Iqalugaarjuit	16/09/11	648	198	72	M	<u> </u>	<0.5	N	I
$\begin{tabular}{ l   l   l   l  l  l  l  l  l  l  l  l $	Iqalugaarjuit	16/09/11	649	258	192	F	<u> </u>	0.5	N	I
$\begin{tabular}{ l l l l l l l l l l l l l l l l l l l$	Iqalugaarjuit	16/09/11	650	201	69	F	<u> </u>	<0.5	N	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	651	202	85	F	<u> </u>	<0.5	N	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	652	163	49	F	<u> </u>	<0.5	N	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	653	182	71	M	<u> </u>	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	654	167	45	M	<u> </u>	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	655	190	68	M	<u> </u>	<0.5	Υ	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	656	206	72	M	I	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	657	192	69	F	I	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	658	245	129	F	<u> </u>	0.5	N	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	659	201	79	F	<u> </u>	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	660	134	49	M	<u> </u>	<0.5	<u>N</u>	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	661	163	47	M	<u> </u>	<0.5	N	I
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	662	204	85	M	M	1.5	Y	R
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	663	541	1906	F	M	389.0	<u>N</u>	Α
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	664	533	1802	F	M	356.0	<u>N</u>	Α
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	665	532	1896	F	M	268.5	N	Α
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	666	182	55	M	<u> </u>	<0.5	Υ	R?
İqalugaarjuit         16/09/11         668         180         57         F         I         <0.5         Y           İqalugaarjuit         16/09/11         669         189         60         M         I         <0.5	Iqalugaarjuit	16/09/11	667	196	67	F	<u> </u>	<0.5	Y	R
İqalugaarjuit         16/09/11         669         189         60         M         I         <0.5         Y           İqalugaarjuit         16/09/11         670         189         66         M         I         <0.5	Iqalugaarjuit	16/09/11	668	180	57	F	<u> </u>	<0.5	Y	I
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Iqalugaarjuit	16/09/11	669	189	60	M	<u> </u>	<0.5	Υ	I
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Iqalugaarjuit	16/09/11	670	189	66	M	<u> </u>	<0.5	<u>N</u>	I
Iqalugaarjuit         16/09/11         672         485         1128         M         M         33.5         N           Iqalugaarjuit         17/09/11         673         244         127         M         I         <0.5	Iqalugaarjuit	16/09/11	671	657	3532	M	RR	111.5	<u>N</u>	Α
Iqalugaarjuit         17/09/11         673         244         127         M         I         <0.5         N           Iqalugaarjuit         17/09/11         674         183         56         F         I         <0.5	Iqalugaarjuit	16/09/11	672	485	1128	M	M	33.5	<u>N</u>	Α
Iqalugaarjuit         17/09/11         674         183         56         F         I         <0.5         N           Iqalugaarjuit         17/09/11         675         192         67         F         I         <0.5	Iqalugaarjuit	17/09/11	673	244	127	M	<u> </u>	<0.5	<u>N</u>	I
Iqalugaarjuit         17/09/11         675         192         67         F         I         <0.5         Y           Iqalugaarjuit         17/09/11         676         182         54         F         I         <0.5	Iqalugaarjuit	17/09/11	674	183	56	F	<u> </u>	<0.5	N	I
Iqalugaarjuit         17/09/11         676         182         54         F         I         <0.5         Y           Iqalugaarjuit         17/09/11         677         455         1035         M         R         0.5         Y           Iqalugaarjuit         17/09/11         678         590         2248         F         M         430.0         N	Iqalugaarjuit	17/09/11	675	192	67	F	<u> </u>	<0.5	Y	I
Iqalugaarjuit         17/09/11         677         455         1035         M         R         0.5         Y           Iqalugaarjuit         17/09/11         678         590         2248         F         M         430.0         N	Iqalugaarjuit	17/09/11	676	182	54	F	<u> </u>	<0.5	Y	I
lgalugaarjuit 17/09/11 678 590 2248 F M 430.0 N	Iqalugaarjuit	17/09/11	677	455	1035	M	R	0.5	Y	Α
	Iqalugaarjuit	17/09/11	678	590	2248	F	M	430.0	N	Α

## Appendix G Raw data: stable isotope analysis

Stable isotope analysis results for Arctic char muscle tissue. Samples were analyzed in bulk (B; i.e. not lipid extracted), and lipid extracted (LE) forms, and every twelfth sample was run in triplicate (B, C) to calculate precision. The C:N was calculated as %C/%N. Sample IDs represent the Arctic char individuals captured from four study systems in the Cumberland Sound region from 2002 to 2011; the first two numbers indicate the year of capture (e.g. 0232 was captured in 2002).

Sample	Bulk							Lipid Extracted							
ID	δ <sup>13</sup> C	%С	δ <sup>15</sup> N	%N	C:N	δ <sup>34</sup> S	%S	δ <sup>13</sup> C	%C	δ <sup>15</sup> N	%N	C:N	δ <sup>34</sup> S	%S	
0232	-21.43	50.51	14.54	11.68	4.32			-20.35	41.64	15.02	12.36	3.37			
0233	-20.73	49.57	15.10	12.69	3.91			-20.31	41.37	15.67	12.15	3.40			
0234	-20.79	52.20	14.90	11.24	4.64			-19.65	42.26	15.54	12.33	3.43			
0235	-21.00	50.82	14.21	12.75	3.98			-20.26	42.22	14.46	12.61	3.35			
0236	-21.75	50.48	13.43	12.01	4.20			-20.67	42.23	13.88	12.63	3.34			
0238	-23.17	55.15	14.47	9.32	5.92			-20.79	43.12	14.57	12.44	3.47			
0238 B	-23.09	55.39	14.24	9.56	5.80								••••••		
0238 C	-23.15	55.00	14.17	9.21	5.97				••••••	••••••	•••••		••••••		
0239	-21.78	53.03	14.17	10.68	4.96			-20.06	42.63	14.76	12.73	3.35			
0240	-22.23	54.27	15.35	9.74	5.57			-20.24	42.79	15.81	12.55	3.41			
0240 B		÷ ··= ·						-20.30	42.16	15.99	12.27	3.44			
0240 C								-19.99	42.36	15.58	12.65	3.35			
0241	-24.33	63.73	14.54	5.54	11.51			-20.79	46.85	15.06	12.27	3.82	••••••		
0243	-24.11	62.24	13.30	8.87	7.02			-21.06	45.24	15.16	12.91	3.50	••••••		
0244	-21.32	65 14	14 05	16.00	4 07			-21 26	45.39	13.84	12 99	3 49			
0245	-20.30	51.00	14.18	14.41	3.54			-19.59	42.14	13.74	12.47	3.38			
0248	-22.58	44.41	14.67	9.35	4.75			-20.34	43.51	14.47	12.98	3.35			
02145	-22.58	54.35	13.47	9.78	5.56			-20.50	44.59	14.40	13.01	3.43			
02151	-22.03	42.15	13.96	9.17	4.59			-20.52	44.78	14.29	13.25	3.38	••••••		
02152	-19.81	48.15	13.06	13.65	3.53			-20.53	45.37	14.51	12.93	3.51			
02154	-22 19	52 47	14 46	10.60	4 95			-20.69	42.08	15 17	12 20	3 45			
02155	-21.56	50.24	13.75	12.27	4.10			-20.96	42.12	14.08	12.43	3.39			
02156	-24.24	57.71	14.07	6.90	8.36			-21.10	46.51	14.85	12.61	3.69			
02156 B	-24.40	56.06	13.89	6.53	8.59										
02156 C	-24.35	56.11	13.94	6.55	8.56					•••••	•••••				
02157	-22.26	52.75	13.11	11.02	4.79			-20.57	43.10	14.11	12.96	3.33			
02159	-20.44	48.65	13.56	13.71	3.55			-20.37	41.67	14.38	12.54	3.32	••••••		
02159 B								-20.46	42.50	14.46	12.80	3.32	••••••		
02159 C								-20.30	42.28	14.37	12.71	3.33	••••••		
02164	-20.79	49.08	14.09	12.77	3.84			-20.41	44.31	14.74	13.19	3.36			
02165	-22.35	52.36	13.63	11.27	4.65			-20.57	43.03	14.17	12.75	3.38			
02167	-21.48	51.15	13.78	11.61	4.40			-20.43	43.35	13.79	12.91	3.36			
02168	-21.19	50.13	13.39	12.98	3.86			-20.57	40.71	13.90	11.94	3.41			
02169	-20.84	50.33	14.75	12.26	4.11			-20.23	44.43	15.46	13.01	3.41			
02172	-23.37	56.49	14.22	7.92	7.13			-20.49	45.50	14.91	12.59	3.61	•••••		
02173	-21.10	50.86	14.08	12.24	4.16			-20.05	41.90	14.57	12.28	3.41			
02175	-22.69	53.62	13.57	10.10	5.31			-20.71	44.75	13.88	12.71	3.52			
02176	-21.61	50.14	13.79	12.31	4.07			-20.99	45.37	13.91	13.01	3.49			
02289	-20.99	52.66	14.86	11.30	4.66			-19.51	42.57	15.36	12.71	3.35	•••••		
02290	-19.99	49.56	15.09	13.14	3.77			-19.75	42.10	15.54	12.60	3.34			
02291	-20.41	50.44	15.19	11.94	4.23			-19.62	43.03	15.79	12.62	3.41	•••••		
02292	-21.02	53.45	14.95	10.59	5.05			-19.23	42.75	15.60	12.71	3.36	••••••		
02293	-21.83	55.60	14.88	9.97	5.58			-19.49	42.43	15.49	12.69	3.34	••••••		
02294	-19.76	50.00	14.62	13.07	3.83			-19.67	43.95	15.29	12.67	3.47	••••••		
02295	-20.13	50.36	15.25	12.66	3.98			-19.66	40.55	15.53	12.09	3.35	••••••		
02296	-22.41	57.72	14.30	8.15	7.09			-19.47	42.20	15.05	12.35	3.42	•••••		
02296 B	-22.33	57.46	14.31	8.49	6.77					••••••	••••••		•••••		
02296 C	-20.12	50.72	15.39	12.08	4.20		-	-							
02297		-		-	-			-19.44	42.91	15.77	12.66	3.39			
02298	-20.20	49.11	15.80	11.91	4.12			-19.54	42.29	16.37	12.60	3.36			
02299	-20.77	51.78	14.98	11.14	4.65			-19.68	42.92	15.57	12.37	3.47			
02299 B								-19.54	41.77	15.49	12.46	3.35			
02299 C								-19.54	42.35	15.59	12.77	3.32			
02301	-19.04	46.55	15.58	13.67	3.41	-	-	-19.14	41.58	15.78	12.57	3.31			
02318	-20.70	51.44	15.51	10.98	4.69	-	-	-19.45	42.45	15.90	12.44	3.41			
02319	-20.22	50.52	15.01	12.56	4.02			-19.64	41.18	15.27	12.45	3.31			
02320	-20.92	51.68	14.81	11.44	4.52			-19.63	41.51	15.43	12.35	3.36			
02321	-20.93	50.86	13.73	11.73	4.34			-20.08	41.10	14.30	12.22	3.36			
02323	-20.06	50.58	14.65	12.20	4.14			-19.20	37.65	15.04	11.25	3.35			
02324	-19.97	49.28	15.81	12.74	3.87			-19.79	39.23	15.63	11.60	3.38			
02325	-19.80	49.92	14.19	13.16	3.79			-19.48	40.97	14.54	12.45	3.29			
02326	-21.39	54.52	15.11	9.87	5.52			-19.25	41.18	15.35	12.25	3.36			
02327	-20.48	50.22	14.33	11.62	4.32			-19.51	42.12	14.63	12.50	3.37			
02328	-19.88	49.58	14.67	12.87	3.85			-19.56	40.33	15.01	12.00	3.36			
02328 B	-19.92	49.55	14.72	12.97	3.82										
02328 C	-19.89	49.98	14.64	13.03	3.84					••••••	••••••				
02329	-19.42	48.77	14.52	13.11	3.72			-19.19	42.28	15.01	12.68	3.34			
02330	-19.98	49.06	14.16	13.33	3.68			-19.89	42.18	14.51	12.59	3.35			

02330 B						-19.85	41.64	14.15	12.43	3.35				
02330 C	10.50	40.17	15 50	12 47	2 65	-19.98	41.93	14.31	12.54	3.34				
02331	-19.59	49.17	10.00	13.47	3.00	-19.49	41.00	15.04	12.41	3.37				
02332	-20.31	49 74	14.65	13.06	3.81	-19.30	42.39	15.02	12.72	3.34				
02335	-20.50	50.06	14.73	12.49	4.01	-19.87	42.51	15.21	12.69	3.35				
02336	-20.02	50.28	14.38	12.68	3.96	-19.48	41.30	14.71	12.38	3.34				
02337	-19.50	48.46	15.25	13.89	3.49	-19.73	41.88	15.37	12.48	3.36				
02338	-20.58	50.81	15.39	11.74	4.33	-19.66	41.27	15.64	12.09	3.41				
02339	-20.54	51.32	15.43	11.60	4.42	-19.50	41.56	15.96	12.18	3.41				
02341	-19.85	48.94	15.39	13.07	3.74	-19.59	42.06	15.49	12.52	3.36				
02342	-22.22	29.99	14.67	11.23	0.23	-19.65	42.52	15.28	12.59	3.38				
02343 B	-72.26	55 64	14.01	8.82	6 31	-13.33	42.14	13.17	12.21	5.50				
02343 C	-22.14	55.04	14.54	9.02	6.11									
02344	-20.09	50.24	15.05	11.87	4.23	-19.62	42.15	15.35	12.21	3.45				
02345	-20.03	49.17	14.12	12.94	3.80	-19.66	41.90	14.43	12.59	3.33				
02345 B						-19.66	41.78	14.38	12.42	3.36				
02345 C						-19.71	42.25	14.58	12.77	3.31				
02346	-19.59	50.04	16.05	12.43	4.03	-19.32	41.07	16.35	11.94	3.44				
02347	-20.40	49.75	14.01	13.10	3.70	-20.22	40.99	15.28	12.13	3.30				
02349	-20.39	51 01	15 23	12.09	4 22	-19.62	41 85	15.20	12.02	3.35				
02352	-19.85	49.91	14.34	12.63	3.95	-19.44	41.27	14.75	12.16	3.39				
03531	-22.22	53.23	13.58	11.77	4.52	-20.53	40.66	14.34	12.10	3.36				
03531 B						-20.59	40.77	14.37	12.09	3.37				
03531 C						-20.63	41.30	14.30	12.22	3.38				
03532	-23.51	56.00	14.35	8.80	6.36	-20.64	44.05	15.00	11.96	3.68				
03534	-22.00	48.82	14.25	10.19	4.79	-20.25	43.39	14.73	12.68	3.42				
03537	-21.04	49.10	14.00	10.94	5 30	-20.20	41.90	14.07	12.40	3.30				
03538	-21.55	51 19	13.74	12.06	4 24	-20.35	42.07	14.38	12.50	3 44				
03539	-21.34	50.69	13.91	12.63	4.01	-20.42	41.82	14.63	12.50	3.35				
03540	-22.47	52.20	14.05	11.12	4.69	-20.70	40.76	14.61	11.98	3.40				
03541	-22.04	54.09	14.63	10.41	5.19	-20.09	41.36	15.23	12.11	3.42				
03542	-22.97	55.53	14.40	9.34	5.95	-20.03	44.33	14.88	12.78	3.47				
03668	-22.81	55.72	14.80	9.19	6.06	-20.06	43.99	15.37	12.94	3.40				
03670	-20.16	47.99	14.49	13.50	3.55	-19.83	42.07	15.02	12.93	3.30				
03671 B	-21.94	52.63	14.70	10.01	4.99	-19.92	44.27	15.17	13.07	3.39				
03671 C	-22.01	54.00	14.85	10.43	5.02									
03673	-21.22	51.57	14.39	12.49	4.13	-20.52	46.14	14.78	12.91	3.57				
03673 B	••••••					-20.12	44.54	14.79	12.96	3.44				
03673 C						-20.09	43.77	14.88	12.91	3.39				
03674	-21.13	51.83	14.01	13.24	3.91	-20.58	44.57	14.36	13.12	3.40				
03675	-22.33	53.48	14.54	10.95	4.88	-20.37	44.37	14.76	12.93	3.43				
03680	-21.77	52.04	13.95	11 27	4.29	-20.36	43.80	14.39	13.00	3.30				
03685	-22.00	53 77	14 31	10.65	5.05	-19 59	44 47	14 94	13.04	3 41				
03686	-21.80	51.45	13.72	12.62	4.08	-20.23	44.37	14.24	13.33	3.33				
03688	-20.22	49.99	13.47	13.11	3.81	-19.21	44.75	13.75	13.51	3.31				
03852	-20.03	49.00	13.48	13.60	3.60	-19.83	43.62	13.56	13.01	3.35				
03853	-19.95	49.62	14.13	13.40	3.70	-19.60	43.26	14.49	12.78	3.38				
03854	-19.90	47.58	13.64	13.67	3.48	-19.94	42.20	13.50	12.57	3.36				
03855 B	-19.79	46.37	15.83	14.40	3.22	-20.26	41.59	15.54	12.76	3.26				
03855 C						-20.19	41.62	16.04	12.75	3.20				
03856	-19.64	49.16	14.61	13.99	3.51	-19.84	42.90	14.69	12.65	3.39				
03857	-19.72	48.10	14.92	13.53	3.56	-19.71	40.99	14.97	12.34	3.32				
03859	-20.48	49.92	13.22	12.28	4.06	-19.69	44.00	13.32	13.01	3.38				
03860	-19.91	50.38	14.27	12.52	4.02	-19.53	44.00	14.62	12.39	3.55				
03864	-19.92	51.54	14.95	13.98	3.69	-19.57	42.34	15.55	12.58	3.36				
03865	-20.10	49.67	16.11	13.38	3.71	-19.74	43.95	15.70	13.21	3.33				
03867	-21.00	54.60	13.09	11.01	4.00	-19.56	44.55	12.95	12.70	3.40				
03867 B	-21.41	53.03	14.06	10.80	4.91	10.02	42.04	14.07	12.70	0.00				
03867 C	-21.49	54.26	14.17	11.15	4.87									
03868	-20.23	51.31	13.79	12.59	4.07	-19.38	43.89	14.18	13.01	3.37				
03868 B						-19.22	43.70	14.35	12.98	3.37				
03868 C						-19.45	52.83	14.14	15.77	3.35				
03869	-20.15	51.66	14.67	12.92	4.00	-19.45	43.51	15.35	12.85	3.39				
03871	-19.37	40.20 51.83	15.47	11 32	3.57	-19.37 _10.90	43.29 43.83	15.00	12.97	3.34				
03872	-20.03	49.39	15.20	13.73	3 60	- 19.80 -19.21	43.03	15.73	12.07	3.40				
03873	-20.16	49.86	14.50	12.86	3.88	-19.26	42.94	14.72	12.66	3.39				
03874	-19.74	48.67	14.99	13.73	3.54	-19.67	41.85	15.60	12.55	3.33				
03875	-20.06	50.10	13.91	13.17	3.80	-19.44	43.00	14.18	12.51	3.44				
03876	-21.45	53.53	15.17	10.73	4.99	-19.79	44.75	15.50	12.58	3.56				
03878	-19.95	50.45	13.79	13.05	3.87	-19.33	44.04	15.47	11.18	3.94				
03879	-20.24	48.71	13.65	13.16	3.70	-19.94	42.21	14.29	12.54	3.37				
03880	-19.65	48.78	14.23	13./4	3.55	-19.55	43.21 11 FO	14.64	12.92	3.34 3.2F				
03881 B	-19.83	49.98	14.77	13.49	3.70	-19.37	41.00	10.04	12.40	5.50				
03881 C	-20.00	50.19	14.73	13.40	3.75									
03882	-21.33	52.48	13.41	11.56	4.54	-19.71	44.11	14.03	12.78	3.45				
03882 B						-19.84	42.99	13.74	12.54	3.43				
03882 C								-19.86	43.30	13.78	12.48	3.47		
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03883	-20.46	51.08	13.56	12.16	4.20			-19.26	42.24	13.79	12.52	3.37		
03884	-20.18	50.52	15.23	13.25	3.81			-19.76	42.14	15.64	12.50	3.30		
03886	-20.14	50.97	13 43	13.21	3.78			-19.39	41.24	13.00	12.27	3 35		
03887	-21.29	52.53	13.55	11.44	4.59			-19.71	43.09	13.95	12.79	3.37		
03888	-20.25	51.30	14.84	13.11	3.91			-19.69	43.37	15.37	13.11	3.31		
03889	-20.06	50.15	14.94	13.15	3.81			-19.83	43.36	15.68	12.73	3.41		
03890	-20.23	50.48	13.87	12.96	3.89			-19.58	43.36	14.60	12.92	3.36		
03891	-20.33	50.40	15.32	11.95	4.22			-19.61	43.80	15.70	12.67	3.46		
03892	-20.26	50.72	15.16	12.77	3.97			-19.48	43.58	15.68	13.09	3.33		
03893	-20.31	50.17	13.07	12 71	3.01			-20.24	43.90	14.57	12.09	3.40		
03894 B	-20.35	50.68	13.84	12.68	4.00				-10.17			0.00		
03894 C	-20.28	50.34	13.75	12.90	3.90					•••••	•••••			
04126	-21.09	50.79	13.92	12.71	4.00			-19.69	44.37	14.76	13.28	3.34		
04127	-22.02	51.78	13.47	12.82	4.04			-20.45	44.82	14.39	13.56	3.30		
04128	-22.94	54.22	13.61	9.67	5.61			-20.02	43.05	14.80	12.25	3.52		
04130	-21.64	52.53	13.01	12.28	4.28			-19.75	44.08	14.42	13.43	3.28		
04133	-20.33	49.20	12.80	12.42	3.85			-19.32	43.63	13.39	13.33	3.27		
04136	-22.30	51.63	13.91	10.94	4.72			-20.42	44.61	14.33	13.40	3.33		
04137	-21.42	51.28	13.40	12.68	4.04			-20.36	43.77	13.82	13.08	3.35		
04138	-20.47	48.65	12.92	13.44	3.62			-20.12	43.33	14.07	13.30	3.26		
04138 B								-20.34	43.44	14.02	13.28	3.27		
04138 C	00.50	10.11	40 70	40.54				-20.24	43.58	13.78	13.37	3.26	••••••	
04139	-20.50	49.11 5/ 15	13.79	13.54	5.03		••••••	-19.87	43.04	14.30	13.11	3.20	••••••	
04141	-21.35	50.24	13.15	12.07	4.16			-19.84	43.69	14.29	13.29	3.29		
04141 B	-21.30	51.17	13.38	12.40	4.13					0				
04141 C	-21.27	51.38	13.39	12.62	4.07						•••••			
04142	-21.56	50.26	13.21	12.36	4.07			-20.14	44.42	13.87	13.33	3.33		
04143	-20.80	49.12	13.06	13.16	3.73			-19.91	44.15	13.78	13.52	3.27		
04145	-20.43	49.34	13.33	13.19	3.74			-19.58	44.18	14.19	13.43	3.29		
04140	-21.00	50.31	13.00	11.92	4.33			-20.21	44.59	13.95	13.23	3.37		
04148	-21.34	49.81	13.23	12.96	3.84			-20.25	44.07	13.18	13.33	3.31		
04150	-20.90	50.39	13.49	13.02	3.87			-19.63	43.89	14.17	13.35	3.29		
04169	-21.10	51.21	14.33	12.28	4.17			-19.43	43.26	14.73	13.16	3.29		
04342	-19.93	50.67	14.28	12.83	3.95			-18.88	43.52	14.79	13.29	3.27	••••••	
04343	-19.93	51.84	13.18	12.63	4.11			-18.41	44.78	13.78	13.53	3.31	••••••	
04343 D								-18.57	45.10	13.62	13.00	3.30	••••••	
04344	-20.85	51.80	13.84	11.59	4.47			-19.32	44.61	14.49	13.47	3.31	••••••	
04345	-19.84	49.67	14.34	12.98	3.83			-19.44	43.71	15.19	13.14	3.33	••••••	
04346	-20.96	54.16	14.65	10.77	5.03			-19.32	44.86	15.19	13.41	3.35		
04346 B	-21.12	54.91	14.64	10.91	5.03									
04346 C	-21.15	51.94	14.73	10.14	5.12			10.11	44.04	44.07	40.40			
04348	-21.08	54.25	13.66	10.87	4.99			-19.11	44.94	14.27	13.40	3.36		
04349	-19 73	51.05	13.83	12 49	4 15			-18 73	40.33	14.32	12.06	3.34	••••••	
04351	-20.40	51.68	15.06	12.07	4.28		•••••	-19.26	44.38	15.98	13.11	3.38	••••••	
04352	-19.94	51.18	13.90	12.14	4.22			-19.10	44.82	14.54	13.29	3.37		
04353	-20.27	52.88	13.22	11.75	4.50			-18.90	44.68	13.81	13.42	3.33		
04354	-20.08	50.71	14.29	13.00	3.90			-19.00	44.00	14.83	13.28	3.31		
04355	-19.69	49.76	13.74	13.35	3.73			-19.38	44.62	14.21	13.30	3.35		
04350	-19.79	51.05	13.03	12.04	3.92			-19.12	44.19	14.33	13.42	3.29		
04357 B	20.04	01.04	14.00	12.00	0.00			-19.61	43.88	15.12	13.31	3.30		
04357 C								-19.64	44.27	14.91	13.47	3.29		
04359	-19.98	51.01	12.88	12.92	3.95			-19.41	44.41	13.64	13.43	3.31		
04360	-21.40	53.49	14.53	11.17	4.79			-20.14	44.97	15.51	13.05	3.45		
04361	-19.93	51.31	14.14	12.33	4.16			-19.33	44.13	14.77	13.31	3.31		
04361 C	-19.00	51 48	14.15	12.20	4.15									
04362	-19.62	49.20	13.82	13.68	3.60			-19.79	43.50	14.62	13.26	3.28	••••••	
04363	-19.69	49.36	14.59	13.55	3.64			-19.64	44.41	15.27	13.51	3.29		
04450	-24.73	48.15	8.84	13.52	3.56	6.61	1.01	-24.30	45.02	8.96	13.39	3.36	5.89	1.14
04457	-24.00	48.95	8.90	12.81	3.82	7.90	1.06	-23.86	47.72	9.04	12.71	3.75	7.29	1.27
04458	-22.96	48.25	11.56	13.74	3.51	11.52	1.19	-22.62	40.56	11.86	12.25	3.31	11.31	1.30
04458 LE B								-22.59	42.19	11.82	12.69	3.33		
04463	-23.25	48.59	9.45	13.58	3.58	6.59	1.14	-22.92	45.34	9.83	13.59	3.34	6.48	1.10
04464	-23.61	47.03	8.46	12.34	3.81	5.48	1.01	-23.27	46.14	8.86	13.39	3.44	5.08	1.15
04465	-21.09	47.38	11.89	13.71	3.46	12.15	0.94	-21.42	46.89	12.06	13.25	3.54	11.20	1.12
04479	-21.73	51.95	13.30	12.03	4.32	19.02	0.87	-20.51	45.08	15.18	12.84	3.51	18.45	1.01
04480	-21.36	53.05	13.75	12.09	4.39	18.63	0.81	-20.06	44.46	14.48	11.85	3.75	17.96	0.95
04481	-20.49	49.19	13.46	12.82	3.84	18.26	0.82	-19.95	44.53	14.28	12.82	3.47	17.50	0.98
04484	-21.10	48 85	9.46	13.65	3.90	9.01	0.81	-20.07	44.42	9 75	13 31	3.01	8 27	1 02
04500	-21.09	51.44	13.35	12.74	4.04	18.72	0.82	-19.90	43.57	13.95	12.78	3.41	17.81	1.02
04522	-20.25	50.79	13.15	12.76	3.98	18.65	0.72	-19.24	44.46	13.66	13.11	3.39	17.67	0.91
04523								-20.40	45.64	13.77	13.06	3.50	18.00	1.03
04523 B				- 10									17.94	0.99
04525	-20.82	48.89	9.48	13.64	3.58	15.61	0.99	-19.82	46.44	12.61	13.19	3.52	14.53	1.25
04002	-22.21	03.51	13.17	10.73	4.99	10.20	0.92	-24.40	40.11	10.50	13.70	3.29	9.30	1.14

04532 B	-24.43	48.54	10.37	13.86	3.50	10.07	1.06							
04540	-23.13	56.51	13.73	9.76	5.79			-20.45	45.58	14.20	12.84	3.55	18.01	1.08
04541	-20.80	49.67	14.00	13.47	3.69	17.97	0.99	-20.20	43.92	14.33	13.06	3.36	18.17	0.99
04542	-21.84	52.23	14.73	11.15	4.68	18.08	0.91	-19.97	45.18	15.23	13.29	3.40	18.19	1.10
04548	-21.28	51.22	14.65	12.37	4.14	18.46	1.08	-20.24	44.46	14.97	12.84	3.46	17.92	1.05
04548 B						18.16	1.07						18.57	1.11
04584	-25.90	48.88	9.94	14.12	3.46	4.79	1.36	-25.79	46.18	9.85	13.33	3.46		
04587	-25.34	50.18	11.09	13.59	3.69	18.16	0.68	-24.89	45.95	10.94	13.47	3.41		
04588	-25.54	48.62	9.54	13.71	3.55	5.58	1.34	-25.72	47.39	9.49	12.67	3.74		
04589	-26.09	48.82	9.43	13.84	3.53	3.24	1.20	-26.16	46.75	9.36	13.22	3.54	••••••	
04595	-26.43	46.25	9.95	13.35	3.46	1.70	1.42	-26.27	44.86	9.74	13.45	3.34		
04602	-26.62	50.17	10.18	13.00	3.86	6.05	1.19	-25.58	45.78	10.12	13.32	3.44		
04605	-24.21	49.36	11.69	14.21	3.47	5.50	1.32	-24.18	45.04	11.52	13.54	3.33		
04606	-26.10	47.63	10.04	13.37	3.56	2.22	1.27	-25.82	45.64	9.98	13.49	3.38		
								-25.71	45.21	10.12	13.44	3.36		
	~~ ~~	=						-25.74	45.26	10.09	13.35	3.39		
04610	-27.70	50.48	8.29	13.53	3.73	4.07	1.11	-27.13	46.16	8.18	13.37	3.45	••••••	
04612	-25.99	49.28	9.73	13.72	3.59	6.55	1.19	-26.03	47.88	9.46	13.10	3.66	••••••	
04625	-22.09	48.06	9.02	14.17	3.39	6.37	1.17	-21.99	42.69	9.03	13.07	3.27	••••••	
04625 B	25.20	10 22	10.22	12 02	2 50	0.40	1.18	25.20	44.02	10.21	12 /7	2 22	••••••	
04020	-20.39	40.32	0.49	12.02	2.50	4.40 E 06	1.10	-23.20	44.92	0.42	13.47	3.33	•••••	
04027 04627 P	-20.37	40.1Z	9.40	10.77	3.71	5.00	1.30	-23.04	45.00	9.43	13.30	3.30	••••••	
04627 C	-22.33	53.55	13.05	10.77	4.97								•••••	
04620	-26.30	17 09	10.49	12.00	3.56	4.46	1 22	-25.94	11 22	10.15	13 55	3 27	•••••	
04639	-20.30	47.90	0.61	12 21	3.00	4.40	0.04	-25.04	44.33	0.45	12.00	3.21	••••••	
04030	-10.00	49.10 50.25	12.61	12.00	2.09	17.50	0.94	-10.29	40.07	12.43	12.23	3.20	••••••	
04643	-19.90	40.04	13.01	12.99	3.00	17.00	0.90	-19.20	44.01	13.94	13.30	3.37	••••••	
04043	-13.35	53.56	13.00	11 /6	1.67	17.23	0.75	-19.02	44.41	14.07	12.25	3.04	••••••	
04645	-21.10	61 17	13.30	14 76	4 1/	17.49	0.07	-19.40	44.09	13.02	12.00	3.49		
04646	-20.78	44 70	12 52	10.13	4 4 1	17 51	0.33	-19.46	45 17	14 02	13 10	3 45		
04648	-21 58	56 28	13.06	10.13	5.59	17.59	0.70	-19.55	46.09	13 47	12 84	3 59		
04648 B	21.00	00.20	10.00	10.07	0.00	17 55	0 71	10.00	-0.00	10.77	12.07	0.00	•••••	
04649	-20 57	52.09	13 30	12 40	4 20	17.00	0.87	-19 35	44 32	13.62	13 28	3 34	••••••	
04649 B	20.01	02.00	10.00	12.40	4.20		0.07	-19.66	44.33	13.68	13 18	3.36	••••••	
04649 C					••••••			-19.20	44.22	13.70	13.21	3.35	••••••	
04650	-20 49	48 84	13.08	11 74	4 16	17 16	0.78	-19.44	44 77	13 41	13 13	3 41	••••••	
04651	-20.43	53.46	12.65	11.61	4.61	17.08	0.63	-18.91	44.85	13.16	13.00	3.45	••••••	
04652	-22.57	57.22	12.93	9.13	6.27	17.50	0.68	-19.55	45.14	13.59	13.02	3.47	••••••	
04655	-21.92	56.58	13.46	10.16	5.57	17.67	0.85	-19.32	44.91	13.41	13.08	3.43	•••••	
04659	-20.86	53.36	13.42	11.60	4.60	17.52	0.93	-19.42	44.95	14.04	12.98	3.46		
04661	-20.99	50.55	12.89	12.95	3.90	17.22	0.88	-19.98	44.51	13.47	13.33	3.34		
04663	-20.46	50.56	12.59	12.62	4.01	17.29	0.74	-19.66	44.21	13.09	13.06	3.39	,	
04664	-21.84	57.73	12.62	9.79	5.89			-19.83	47.76	13.12	12.65	3.78		
04665	-21.92	55.09	12.87	10.61	5.19	17.51	0.82	-19.48	45.50	13.12	13.72	3.32		
04666	-20.92	54.36	12.99	11.52	4.72	17.57	0.82	-19.34	46.71	13.40	13.58	3.44		
04666 B						17.54	0.82		•••••		•••••			
04667	-20.61	52.22	13.82	12.15	4.30			-20.04	47.72	14.14	12.92	3.69		
08001	-20.04	51.71	14.09	12.99	3.98			-19.39	44.58	14.48	13.19	3.38		
08003	-21.33	53.65	13.89	10.26	5.23			-19.74	43.09	14.48	12.49	3.45		
08005	-20.08	49.96	13.77	13.41	3.73			-20.13	43.42	14.01	12.96	3.35		
08008	-21.28	56.29	14.49	10.01	5.62			-19.50	44.54	14.86	12.91	3.45		
08009	-19.81	51.14	14.85	12.37	4.13			-19.22	44.71	15.10	13.12	3.41		
08010	-20.21	48.69	14.18	13.64	3.57			-20.33	42.74	14.50	12.77	3.35		
08011	-21.55	56.30	15.37	9.48	5.94			-20.28	45.29	15.70	12.93	3.50		
08013	-20.33	49.72	14.57	13.05	3.81			-20.71	44.11	15.11	13.18	3.35		
08013 B								-20.28	44.75	15.08	13.17	3.40		
08013 C								-20.55	53.57	15.06	15.91	3.37		
08016	-21.15	53.92	16.09	10.13	5.32			-19.87	46.12	16.55	12.98	3.55		
08025	-20.15	50.94	13.89	11.96	4.26			-19.62	43.01	14.32	12.84	3.35		
08027	-20.53	52.34	13.98	12.39	4.23			-19.69	44.75	14.58	13.45	3.33		
08032	-20.70	52.30	14.37	11.90	4.39			-20.09	43.97	14.63	13.12	3.35		
08035	-19.90	49.77	14.69	12.90	3.86			-20.00	42.80	15.13	12.82	3.34	••••••	
08036	-21.28	53.55	15.10	11.21	4.78			-20.49	44.77	15.42	13.16	3.40		
08039	-20.90	51.68	14.14	12.60	4.10			-20.87	42.42	14.63	12.72	3.34		
08042	-20.90	52.41	15.52	11.71	4.48			-20.22	44.69	15.76	13.24	3.37		
08044	-20.08	50.33	14.99	12.86	3.91			-19.84	44.48	15.53	13.28	3.35		
08045	-19.67	49.89	14.37	13.08	3.82			-19.97	41.52	14.56	12.19	3.41		
08050	-20.20	50.37	13.89	13.14	3.83			-20.52	43.33	14.42	12.82	3.38		
08059	-20.05	51.13	14.55	12.99	3.94			-20.50	44.09	14.96	13.22	3.33		
08080	-20.04	50.61	13.89	13.14	3.85			-19.42	44.55	14.14	13.09	3.40		
08080 B								-19.47	44.91	13.90	13.16	3.41	•••••	
08080 C	00.04	FO ^^	45 -0		4 70			-19.34	44.20	13.90	13.05	3.39		
08082	-20.64	52.39	15.72	11.15	4.70			-18.96	44.93	15.81	12.80	3.51		
08085	-20.36	50.54	14.72	12.63	4.00			-19.67	44.82	15.01	13.30	3.37		
08086	-20.58	51.50	14.81	11.80	4.37			-19.44	44.59	14.85	13.10	3.40	••••••	
08003	-20.33	51.30	14.52	11.01	3.94			-19.64	44.44	14.54	13.12	3.39	•••••	
08002 P	-20.03	51.90	15.05	11.79	4.40			-19.09	44.05	15.09	13.02	3.30	•••••	
08002 0								-20.48	44.14	15.51	13.15	3.30	•••••	
08102	-20 24	50.22	11 51	12 10	2 0 4			-10.60	44.30	14 50	13.01	3.41 2.25	•••••	
00103	-20.24	50.33	14.51	10.10	3.84			-19.60	44.44	14.59	13.20	3.35		
08107	-20.09	18 04	14.04	13 22	4.14			-19.90	44.09	14.00	12.31	3.30	••••••	
08100	- 19.00	40.94 50 60	1/ 50	10.32	3.07			-19.00	43.01	1/ 62	12.71	2 20		
08110	-20.30	50 97	14.02	13.73	3.90			-19.04	44.33	14.03	13.10	3.39	•••••	
08120	-19.03	53.67	15.03	11 /3	J.00 4 60			-10.94	43 58	15 08	12.13	3.39	•••••	
00120	20.01	00.04	10.00	11.75	4.03			10.00	-0.00	10.00	12.00	5.77		

08134	-21.80	57.75	15.19	9.15	6.32	-19.82 46.84 15.24 12.37 3.79	
08135	-19.23	49.56	14.46	13.40	3.70	-18.82 44.84 14.61 13.05 3.44	
08136	-20.19	52.05	14.60	12.13	4.29	-19.20 45.12 14.67 12.88 3.50	
08140	-20.51	52 57	15 35	12 11	4.34	-19.52 41.10 15.75 12.01 3.42	
08140 B		02.01				-19.40 42.04 15.57 12.26 3.43	
08140 C						-10.51 42.05 15.50 12.41 3.47	
08140 0	-20.04	51 63	1/ /7	11 55	4 47		
00141	20.34	50.70	14.47	12.06	2 00		
00145	-20.29	40.01	14.00	10.00	0.09		
00140	-20.22	49.01	13.71	10.17	3.11	-19.71 44.23 14.22 15.57 5.20	
00151	-19.74	50.15	14.47	13.12	3.82	-19.16 44.52 14.75 13.62 3.27	
08151 B						-19.56 45.98 14.54 13.49 3.41	
08151 C						-19.30 44.47 14.64 13.45 3.31	
08156	-19.42	49.22	14.67	13.92	3.54	-19.28 44.47 13.71 12.77 3.48	
08158	-20.18	50.44	15.23	12.53	4.03	-19.43 44.57 13.27 13.00 3.43	
08162	-20.34	51.84	14.95	11.93	4.34	-19.03 45.18 13.44 12.97 3.48	
08174	-20.91	52.82	14.67	11.00	4.80	-19.38 42.34 14.32 13.22 3.20	
08181	-19.57	47.95	14.47	13.32	3.60	-19.58 44.11 14.61 13.07 3.37	
08182	-21.42	53.31	14.75	10.58	5.04	-19.90 45.59 15.24 12.98 3.51	
11001	-18.95	47.37	15.42	13.61	3.48	-18.50 45.63 15.66 14.42 3.16	
11010	-19.19	46.53	15.24	13.93	3.34	-18.96 45.24 15.34 14.34 3.15	
11012	-19.37	49.09	15.17	13.69	3.58	-18.74 45.41 15.40 14.17 3.20	
11015	-19.95	49.01	14.27	13.41	3.65	-19.18 45.22 14.59 14.18 3.19	
11025	-19.91	50.98	13.92	12.63	4.04	-18.64 45.46 14.22 14.16 3.21	
11030	-19.92	50.87	15.03	12.43	4.09	-18.54 45.78 15.26 14.29 3.20	
11036	-19.41	47.68	14.52	14.22	3.35	-19.27 45.22 14.84 14.41 3.14	
11036 B	-19.41	47.46	14.58	14.17	3.35		
11036 C	-19.35	47.81	14.48	14.29	3.35		
11039	-19.74	49.05	14.45	13.23	3.71	-18.81 46.17 14.90 14.61 3.16	
11057	-18.92	46.63	14.15	13.67	3.41	-18.62 45.25 14.57 14.28 3.17	
11058	-19.24	49.24	16.48	12.65	3.89	-18.12 45.74 16.81 14.26 3.21	
11060	-19.02	47.33	15.47	13.58	3.49	-18.70 45.42 16.05 14.65 3.10	
11068	-19.81	49.88	14 61	12 75	3.91	-18 63 46 20 15 01 14 41 3 21	
11068 B		.0.00			0.01	-18.68 45.75 15.13 14.40 3.18	
11076	-19 39	48 75	14 75	13 20	3 69	-18 74 45 46 14 85 13 97 3 25	
11088	-20.66	51 22	15.64	11 33	4 52	-18 75 45 70 15 96 14 08 3 25	
11101	_10.00	47.04	15.07	14.06	2 /1		
11101	-10.21	47.94	15.02	12.00	2 21	-10.00 45.07 15.11 14.15 5.15	
11104	10.22	40.03	15.01	10.92	2.01		
11109	-19.32	40.04	15.05	12.00	3.00		
11110	-19.40	40.39	10.44	10.01	3.00	-10.92 40.27 10.76 14.24 0.10	
11119	-19.54	49.35	14.00	12.99	3.80	-18.45 45.96 14.92 14.35 3.20	
11119 B	-19.44	49.27	14.63	13.10	3.76		
11119 C	-19.48	49.11	14.65	13.09	3.75		
11124	-19.47	46.17	15.15	13.72	3.37	-19.31 44.69 15.30 14.17 3.15	
11127	-19.23	46.95	15.13	13.72	3.42	-18.92 45.32 15.39 14.21 3.19	
11128	-20.21	51.71	13.04	11.48	4.51	-18.31 45.67 13.42 14.12 3.23	
11131	-19.71	47.02	15.17	13.40	3.51	-19.33 45.64 15.76 14.25 3.20	
11134	-19.12	47.26	14.68	13.33	3.55	-18.69 45.49 15.19 14.30 3.18	
11134 B						-18.61 45.40 15.16 14.25 3.18	
11134 C						-18.68 45.71 15.24 14.16 3.23	
11136	-19.26	47.91	15.08	13.35	3.59	-18.61 45.66 15.44 14.55 3.14	
11138	-19.86	46.87	14.81	13.09	3.58	-19.08 44.47 15.15 14.10 3.15	
11138 B						-19.14 45.60 15.17 14.43 3.16	
11138 C						-19.11 45.99 15.09 14.52 3.17	
11141	-18.84	46.82	16.06	13.45	3.48	-18.51 44.90 16.21 14.38 3.12	
11155	-19.49	47.86	14.43	13.67	3.50	-18.76 45.16 15.05 14.43 3.13	
11163	-19.83	46.89	15.06	13.32	3.52	-19.09 45.73 15.50 14.44 3.17	
11169	-19.25	46.36	15.02	13.27	3.49	-18.73 45.93 15.31 14.69 3.13	
11173	-19.39	48.16	14.69	12.89	3.74	-18.43 45.80 15.25 14.45 3.17	
11173 B	-19.38	47.81	14.84	12.90	3.71		•••••
11176	-19.38	48.23	16.03	13.27	3.64	-18.72 44.74 16.39 14.13 3.17	•••••
11180	-19.54	48.55	15.51	11.91	4.08	-18.11 45.42 15.68 14.30 3.18	
11182	-19.28	47.16	14.43	13.19	3.58	-18.67 45.31 14.74 14.36 3.16	
11184	-19.44	48.18	15.38	13.12	3.67	-18.78 45.71 15.70 14.53 3.15	
11186	-19.27	42.11	14.96	12.25	3.44	-18.86 45.57 15.57 14.29 3.19	•••••
11189	-19.41	46.69	14.83	13.14	3.55	-18.76 46.25 14.85 14.62 3.16	
11189 B						-18.88 42.96 15.31 13.82 3.11	
11189 C	••••••					-18 65 45 28 14 97 14 47 3 13	
11192	-19 46	47 22	15 26	12 81	3.68	-18.62 45.35 15.50 14.34 3.16	
11202	-20.06	49.02	14 07	12.06	4.06	-18 90 45 40 14 56 14 39 3 16	
11203	-19 72	49.29	13.69	12.65	3.90	-18.66 45.14 14.21 14.45 3.12	
11205	-13.12		1/ 60	12.00	1 06	-10.00 -11.21 14.43 3.12	
11203	-20.00	/0.74	12 55	12.14	3 27	-10.00 +0.10 14.70 14.00 0.10 -18.36 //5.// 13.7/ 4//64 0.40	
11207	-19.44	49.71	14 20	12.00	3.01	-10.00 40.44 10.74 14.01 3.13	
11200	-19.67	40.99	14.39	10.02	3.40	-19.33 44.70 14.02 14.07 3.05	
11208 B	-19.67	40.98	14.49	13.79	3.41		
11208 C	-19.66	40.54	14.54	13.56	3.43	40.20 AF CF 44.04 44.54 0.44	
11214	-20.41	48.73	14.33	12.84	3.80	-19.39 45.65 14.81 14.54 3.14	
11217	-20.61	52.09	14.13	11.02	4.73	-18.55 45.04 14.40 14.26 3.16	
11005			1/1 92	11.21	4.73	-18.60 45.37 15.18 14.41 3.15	
11220	-20.65	52.99	14.02				
11220 11225	-20.65 -20.04	52.99 50.21	14.57	12.32	4.07	-18./1 45.15 14.89 14.23 3.1/	
11220 11225 11227	-20.65 -20.04 -19.52	52.99 50.21 45.16	14.57 15.29	12.32 13.63	4.07 3.31	-18.71 45.15 14.89 14.23 3.17 -19.22 44.58 15.54 14.54 3.07	
11220 11225 11227 11238	-20.65 -20.04 -19.52 -19.74	52.99 50.21 45.16 49.28	14.57 15.29 15.26	12.32 13.63 13.00	4.07 3.31 3.79	-18.71 45.15 14.89 14.23 3.17 -19.22 44.58 15.54 14.54 3.07 -18.78 44.81 15.81 14.12 3.17	
11220 11225 11227 11238 11238 B	-20.65 -20.04 -19.52 -19.74	52.99 50.21 45.16 49.28	14.57 15.29 15.26	12.32 13.63 13.00	4.07 3.31 3.79	-18.71 45.15 14.89 14.23 3.17 -19.22 44.58 15.54 14.54 3.07 -18.78 44.81 15.81 14.12 3.17 -18.78 45.16 15.60 14.31 3.16	
11220 11225 11227 11238 11238 B 11238 C	-20.65 -20.04 -19.52 -19.74	52.99 50.21 45.16 49.28	14.02 14.57 15.29 15.26	12.32 13.63 13.00	4.07 3.31 3.79	-18.71 45.15 14.89 14.23 3.17 -19.22 44.58 15.54 14.54 3.07 -18.78 44.81 15.81 14.12 3.17 -18.78 45.16 15.60 14.31 3.16 -18.80 43.22 15.82 13.72 3.15	
11220 11225 11227 11238 11238 B 11238 C 11239	-20.65 -20.04 -19.52 -19.74 -20.69	52.99 50.21 45.16 49.28 51.90	14.02 14.57 15.29 15.26 14.25	12.32 13.63 13.00 11.63	4.07 3.31 3.79 4.46	-18.71 45.15 14.89 14.23 3.17   -19.22 44.58 15.54 14.54 3.07   -18.78 44.81 15.81 14.12 3.17   -18.78 45.16 15.60 14.31 3.16   -18.78 45.16 15.60 14.31 3.16   -18.80 43.22 15.82 13.72 3.15   -18.75 45.67 14.81 14.28 3.20	

11245	-19 57	47 55	14 14	13 91	3.42	-19 10	45 38	14.63	14 33	3 17	
11248	-19 14	47 14	15.84	13 17	3 58	-18 59	44 94	16 24	14 42	3.12	
11249	-19.80	48 89	14 22	12.92	3 78	-18.88	45.31	14 47	14 29	3 17	
11250	-20.64	54 76	14 16	10.06	5 45	-17.56	45 40	13.98	14.33	3 17	
11250 B	-19 52	51.62	13 51	11 85	4 36	17.00	-1010	10.00	14.00	0.17	
11250 C	-19.78	51.02	13 57	11 52	4 51		••••••				
11254	-20 20	49.51	13.80	12.08	4 10	-18 68	46 17	14 21	14 47	3 19	
11268	-19.67	48 76	13 26	13.05	3.74	-18 71	46.22	13.88	14 55	3.18	
11278	-21 01	52.46	15 25	10.00	4 96	-18.82	45 59	15.81	14 14	3.22	
11270	-21.01	53.68	1/ 86	10.07	5.01	-18.67	45.60	15.01	1/ 30	3.17	
11273	-10.06	47.95	12.66	12.22	3.60	-10.07	45.01	14 22	14.33	3.16	
11204	-10.70	47.05	15.00	12.20	3.00	-19.20	45.47	14.52	14.41	3.10	
11302 11202 B	-13.70	40.05	13.10	12.07	3.04	-10.57	45.00	15.39	14.40	3.17	
11302 D						-10.57	45.00	15.40	14.43	2 1 4	
11302 0	-10.61	11 95	14 56	14 41	2 1 1	-10.03	43.20	14 71	14.57	3.14	
11324	-10.79	44.00	14.50	12.01	3.11	-19.25	44.34	14.71	14.01	3.05	
11345	-10.92	40.00	12.49	13.01	3.75	-10.50	45.75	12.01	14.40	3.10	
11340	-19.02	40.23	14 20	13.30	2.00	-19.12	45.77	14.61	14.52	3.13	
11331	-19.40	47.01	12.02	13.04	3.40	- 19.05	40.00	14.01	14.04	3.14 2.10	
11304	-19.04	40.77	15.02	12.90	3.70	- 10.40	40.57	14.50	14.04	3.10	
11357	-19.43	40.30 50.39	1/ 22	12.09	3.39	- 10.09	40.04	14.80	14.50	3.14	
11357	10.60	47.00	15 21	12.23	9.76	- 10.95	40.20	14.05	14.54	2.10	
11000 11050 D	-19.09	47.90	15.51	12.74	3.70	-10.71	45.99	15.57	14.50	3.10	
11300 D	-19.00	40.20	15.54	12.00	3.03						
11330 0	-19.71	47.94	14.16	12.04	3.73	19.90	42.07	14.60	12 50	2 1 0	
11304	-19.57	40.00	14.10	10.04	3.49	-10.09	42.97	14.00	13.50	3.10	
11300	-19.91	49.09	14.00	12.90	3.00	-10./1	40.19	14.40	14.31	3.19 2.15	
11307	- 19.03	47.31	14.24	10.47	3.33	-18.95	40.01	14.05	14.03	3.10	
11300	-20.32	JZ.95	14.02	12.22	4.33	-18.67	40.48	14.35	14.41	3.23	
11300 8						-18.65	40.40	14.39	14.45	3.22	
11272	_20.20	10 75	1151	10 50	2 07	-10.09	40.00	14.33	14.51	3.21	
11372	-20.39	49.70	14.04	12.00	3.91	-10.92	40.00	14.92	14.00	3.10	
11300	- 19.98	49.23	14.01	13.20	3.11	-18.98	45.97	14.42	14.30	3.20	
11301	- 19.30	40.53	14.54	10.07	3.30	-18.49	40.19	14.95	14.39	3.21	
11602	-25.35	40.33	9.78	13.57	3.41	-24.86	45.50	10.08	14.18	3.21	
11603	-26.52	47.75	8.89	13.19	3.62	-25.62	45.96	9.40	14.12	3.26	
11603 B						-25.60	45.89	9.34	14.14	3.25	
11603 C						-25.56	46.19	9.28	14.26	3.24	
11604	-26.47	47.13	9.35	13.43	3.51	-25.75	45.38	9.88	14.40	3.15	
11607	-20.75	48.73	11.94	12.80	3.81	-19.89	46.03	12.38	14.03	3.28	
11608	-19.71	47.68	14.70	13.73	3.47	-19.29	45.85	14.81	14.46	3.17	
11609	-20.41	50.55	14.41	12.88	3.92	-19.01	44.89	14.86	14.28	3.14	
11610	-21.28	48.28	13.65	13.98	3.45	-20.43	45.42	13.93	14.46	3.14	
11611	-19.88	50.33	14.94	12.95	3.88	-18.28	45.77	15.25	14.60	3.13	
11611 B	-19.77	50.16	14.85	13.02	3.85		••••••				
11611 C	-19.74	51.39	14.91	13.21	3.89						
11612	-20.31	51.66	16.02	12.32	4.19	-18.41	45.91	16.50	14.48	3.17	
11613	-20.84	50.71	14.98	11.95	4.24	-18.74	45.45	15.21	14.57	3.12	
11614	-20.75	50.51	15.64	10.60	4.77	-18.45	45.65	16.02	14.56	3.13	
11615	-27.42	48.38	9.09	13.36	3.62	-26.13	45.21	9.49	14.63	3.09	
11616	-20.59	52.22	14.60	12.20	4.28	-18.78	45.81	15.05	14.46	3.17	
11617	-25.83	48.93	9.28	13.68	3.58	-24.88	45.13	9.73	14.50	3.11	
11618	-26.25	49.98	9.74	12.95	3.86	-24.78	45.55	9.82	14.62	3.12	
11618 B						-24.85	46.16	9.77	14.67	3.15	
11618 C						-24.77	45.76	9.79	14.62	3.13	
11621	-22.00	57.03	13.53	10.44	5.46	-18.80	46.71	13.79	14.73	3.17	
11625	-20.84	49.45	11.51	13.26	3.73	-19.73	46.16	11.91	14.72	3.14	
11628	-20.93	50.39	14.25	13.75	3.67	-19.65	46.13	14.71	14.84	3.11	
11630	-21.11	54.08	14.06	11.34	4.77	-18.57	46.48	14.53	14.72	3.16	
11631	-21.01	52.86	14.14	10.74	4.92	-18.51	45.83	14.71	14.71	3.12	
11631 B	-21.28	54.75	14.16	10.50	5.21		••••••				
11631 C	-21.03	53.80	14.48	10.93	4.92	00.55	45.00	10.10	44	0.44	
11632	-24.02	45.81	12.12	13.87	3.30	-23.53	45.26	12.43	14.57	3.11	
11633	-20.94	50.50	14.69	10.93	4.62	-18.54	46.60	15.23	14.74	3.16	
11634	-26.01	47.17	10.17	13.80	3.42	-25.47	45.26	10.24	14.57	3.11	
11635	07 50	47 0 4	0.00	13 42	3.55	-26.70	46.05	8.81	14.75	3.12	
n n ( 117	-27.58	47.64	8.28	40	0.1-	0E E0	4	~ ~~	4 4 0 -	A * A	
11037	-27.58	47.64	8.28 9.64	13.79	3.45	-25.52	45.68	9.82	14.66	3.12	
11637	-27.58 -26.17 -24.79	47.64 47.52 47.50	8.28 9.64 11.91	13.79	3.45 3.32	-23.32 -24.37	45.68 45.87	9.82 12.30	14.66	3.12 3.09	
11637 11638 11639	-27.58 -26.17 -24.79 -21.11	47.64 47.52 47.50 55.43	8.28 9.64 11.91 14.52	13.79 14.33 10.89	3.45 3.32 5.09	-25.52 -24.37 -18.64	45.68 45.87 46.47	9.82 12.30 14.59	14.66 14.85 14.58	3.12 3.09 3.19	
11637 11638 11639 11639 B	-27.58 -26.17 -24.79 -21.11	47.64 47.52 47.50 55.43	8.28 9.64 11.91 14.52	13.79 14.33 10.89	3.45 3.32 5.09	-25.52 -24.37 -18.64 -18.73	45.68 45.87 46.47 45.60	9.82 12.30 14.59 14.68	14.66 14.85 14.58 14.32	3.12 3.09 3.19 3.18	
11637 11638 11639 11639 B 11639 C	-27.58 -26.17 -24.79 -21.11	47.64 47.52 47.50 55.43	8.28 9.64 11.91 14.52	13.79 14.33 10.89	3.45 3.32 5.09	-25.32 -24.37 -18.64 -18.73 -18.66	45.68 45.87 46.47 45.60 46.31	9.82 12.30 14.59 14.68 14.68	14.66 14.85 14.58 14.32 14.42	3.12 3.09 3.19 3.18 3.21	
11637 11638 11639 11639 B 11639 C 11641	-27.58 -26.17 -24.79 -21.11	47.64 47.52 47.50 55.43 51.10	8.28 9.64 11.91 14.52 13.79	13.79 14.33 10.89	3.45 3.32 5.09 4.90	-25.52 -24.37 -18.64 -18.73 -18.66 -18.76	45.68 45.87 46.47 45.60 46.31 46.85	9.82 12.30 14.59 14.68 14.68 14.06	14.66 14.85 14.58 14.32 14.42 14.62	3.12 3.09 3.19 3.18 3.21 3.20	
11637 11638 11639 11639 B 11639 C 11641 11642 11645	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41	47.64 47.52 47.50 55.43 51.10 48.43	8.28 9.64 11.91 14.52 13.79 15.64	13.79 14.33 10.89 10.43 14.02	3.45 3.32 5.09 4.90 3.46	-25.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76	45.68 45.87 46.47 45.60 46.31 46.85 46.26	9.82 12.30 14.59 14.68 14.68 14.06 16.14	14.66 14.85 14.58 14.32 14.42 14.62 14.71	3.12 3.09 3.19 3.18 3.21 3.20 3.15	
11637 11638 11639 11639 B 11639 C 11641 11642 11642	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01	47.64 47.52 47.50 55.43 51.10 48.43 49.97	8.28 9.64 11.91 14.52 13.79 15.64 13.43	13.79 14.33 10.89 10.43 14.02 12.33	3.45 3.32 5.09 4.90 3.46 4.05	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.72 -20.64	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65	14.66 14.85 14.58 14.32 14.42 14.62 14.62 14.71 14.53	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16	
11637 11638 11639 11639 B 11639 C 11641 11642 11643 11644 11644	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27	13.79 14.33 10.89 10.43 14.02 12.33 13.53	3.45 3.32 5.09 4.90 3.46 4.05 3.61	-25.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13	
11637 11638 11639 11639 B 11639 C 11641 11642 11643 11644 11644	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57 -19.89	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50	-25.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.72 -20.64 -19.78 -19.02	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.89	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06	
11637 11638 11639 11639 B 11639 C 11641 11642 11643 11644 11644 11647 11648	-27.58 -26.17 -24.79 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47 48.44	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.50 3.58	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -18.99 -19.02	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.89 14.63	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.56	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12	
11637 11638 11639 11639 B 11639 C 11641 11642 11643 11644 11644 11647 11648 11649 11649 2	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -20.57 -19.89 -19.85 -20.34 -20.54	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.74 9.77	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.89 14.63 13.30	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.55	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17	
11637 11638 11639 11639 11639 11639 11641 11642 11643 11644 11647 11648 11649 11649 11649 11649 11649 11649	-27.58 -26.17 -24.79 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85 -20.34 -20.24 -20.24	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59 49.35 50.00	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26 13.26 13.09	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.74 3.77	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10	9.82 12.30 14.59 14.68 14.68 14.68 14.06 16.14 13.65 13.65 13.89 14.63 13.30	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.55	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.16 3.13 3.06 3.12 3.12 3.17	
11637   11638   11639   11639   11639   11639   11641   11642   11643   11644   11645   11649   11649   11649   11649   11649	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -20.57 -19.89 -19.85 -20.34 -20.24 -20.24 -20.24 -20.24	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59 49.35 50.96	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.12	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26 13.09 12.59	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.74 3.77 4.05	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.76 -19.72 -20.64 -19.78 -19.02 -18.99 -19.17	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.65 13.89 14.63 13.30	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.55	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17	
11637   11638   11639   11639   11639   11639   11641   11642   11643   11644   11645   11646   11647   11648   11649   11649   11649   11649   11649   11649   11649   11650	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -20.57 -19.89 -19.85 -20.34 -20.24 -20.78 -19.84	47.64 47.52 47.50 55.43 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59 49.35 50.96 48.22	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.30 14.08	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26 13.09 12.59 13.29 13.29	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.74 3.77 4.05 3.63 2.52	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.89 14.63 13.30	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.55 14.55	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.15 3.16 3.13 3.06 3.12 3.17 3.17	
11637   11638   11639   11639   11639   11641   11642   11644   11644   11645   11649   11649   11649   11649   11649   11649   11649   11650   11652	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85 -20.34 -20.24 -20.78 -19.84 -19.49	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59 49.35 50.96 48.20 48.20 48.20	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.30 14.08 12.89 14.08	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26 13.09 12.59 13.29 13.29 13.24	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.74 4.05 3.63 3.77 4.05 3.63 3.52	-20.32 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17 -18.94 -18.71 -28.94 -18.71 -20.64	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10 45.38 45.38 44.75	9.82 12.30 14.59 14.68 14.68 14.68 14.06 16.14 13.65 13.65 13.89 14.63 13.30 14.43 13.19	14.66 14.85 14.58 14.32 14.42 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.55 14.48 14.48 14.48	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17 3.17 3.13 3.14 3.14	
11637   11638   11639   11639   11639   11641   11642   11643   11644   11647   11648   11649   11649   11649   11649   11650   11650   11650	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85 -20.34 -20.24 -20.78 -19.84 -19.84 -19.84 -19.84 -20.78	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.90 48.43 49.97 48.90 48.44 49.59 49.35 50.96 48.20 48.43 48.20 48.43	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.30 14.08 12.89 11.92	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.55 13.26 13.09 12.59 13.29 13.74 13.93	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.74 3.77 4.05 3.63 3.74 3.77 4.05 3.63 3.52 3.63	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17 -18.94 -18.71 -23.84 -2	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10 45.38 44.75 45.38 44.75	9.82 12.30 14.59 14.68 14.68 14.68 14.68 14.63 13.65 13.65 13.65 13.89 14.63 13.30	14.66 14.85 14.58 14.32 14.42 14.42 14.62 14.71 14.63 14.27 14.94 14.56 14.55 14.48 14.48 14.24 14.48	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17 3.17 3.13 3.14 3.14 3.12 2.44	
11637   11638   11639   11639   11639   11639   11641   11642   11643   11644   11649   11649   11649   11649   11649   11650   11652   11656   11660	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -20.01 -20.57 -19.89 -19.85 -20.34 -20.24 -20.78 -19.84 -19.49 -24.30 -24.30 -22.61	47.64 47.52 47.50 55.43 51.10 48.43 49.97 48.99 48.47 48.44 49.35 50.96 48.43 48.20 48.43 48.20 48.53	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.30 14.08 12.89 11.92 13.07	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.53 13.84 13.55 13.26 13.09 12.59 13.29 13.74 13.93 13.74	3 45 3.32 5.09 4.90 4.90 3.46 4.05 3.61 3.50 3.58 3.74 3.77 4.05 3.63 3.77 4.05 3.52 3.46 3.52 3.46	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.76 -19.78 -19.02 -18.99 -19.17 -18.94 -18.71 -23.84 -22.32	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.73 45.48 46.75 45.28 44.75 45.12 45.02	9.82 12.30 14.59 14.68 14.68 14.06 16.14 13.65 13.65 13.65 13.89 14.63 13.30 14.43 13.19 12.11 13.19	14.66 14.85 14.58 14.32 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.56 14.56 14.56 14.56 14.48 14.24 14.48	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17 3.17 3.13 3.14 3.12 3.14 3.12	
11637   11638   11639   11639   11639   11639   11641   11642   11643   11644   11645   11646   11649   11649   11649   11649   11650   11656   11660   11662	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85 -20.34 -20.24 -20.78 -19.84 -19.49 -24.30 -22.61 -24.63	47.64 47.50 55.43 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.55 50.96 48.20 48.20 48.20 48.20 48.20 48.20 48.23 48.20	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.12 13.30 14.08 12.89 11.92 13.07 11.44	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.84 13.55 13.26 13.29 13.29 13.74 13.93 13.59 13.76	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.58 3.58 3.58 3.58 3.58 3.58 3.58	-20.32 -24.37 -18.64 -18.73 -18.66 -18.76 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17 -18.94 -18.71 -18.94 -18.71 -23.84 -22.32 -24.12	45.68 45.87 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10 45.38 45.48 46.10 45.38 45.48 45.38 45.42 45.62 45.62	9.82 12.30 14.59 14.68 14.68 14.68 14.68 14.63 13.65 13.89 14.63 13.30 14.43 13.19 12.11 13.19 11.73	14.66 14.85 14.58 14.52 14.42 14.62 14.71 14.53 14.27 14.94 14.56 14.55 14.55 14.48 14.24 14.48 14.24 14.32 14.25	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.16 3.13 3.06 3.12 3.17 3.13 3.14 3.14 3.12 3.14 3.13 3.14 3.13 3.14	
11637   11638   11639   11639   11639   11639   11641   11642   11643   11644   11645   11649   11649   11649   11649   11649   11650   11650   11650   11660   11660   11662   11662	-27.58 -26.17 -24.79 -21.11 -21.25 -19.41 -22.01 -20.57 -19.89 -19.85 -20.34 -20.78 -19.84 -19.49 -20.78 -19.84 -19.49 -22.61 -22.61 -24.63	47.64 47.52 55.43 55.43 51.10 48.43 49.97 48.90 48.47 48.44 49.59 49.35 50.96 48.20 48.43 48.20 48.43 48.20 48.53 48.03	8.28 9.64 11.91 14.52 13.79 15.64 13.43 13.27 13.36 14.18 12.94 13.30 14.08 12.89 11.92 13.07 11.44	13.79 14.33 10.89 10.43 14.02 12.33 13.53 13.53 13.26 13.26 13.29 13.29 13.29 13.74 13.59 13.74 13.59 13.74	3.45 3.32 5.09 4.90 3.46 4.05 3.61 3.50 3.58 3.58 3.58 3.57 3.63 3.63 3.63 3.52 3.46 3.57 3.49	-20.52 -24.37 -18.64 -18.73 -18.66 -18.76 -18.72 -20.64 -19.78 -19.02 -18.99 -19.17 -18.94 -18.71 -23.84 -22.32 -24.12 -24.11 -24.11	45.68 45.67 46.47 45.60 46.31 46.85 46.26 45.97 44.69 45.73 45.48 46.10 45.38 44.75 45.12 45.02 44.62 44.62	9.82 12.30 14.59 14.68 14.68 14.68 14.68 14.68 13.65 13.65 13.65 13.89 14.63 13.30 14.43 13.19 12.11 13.19 11.73 11.64	14.66 14.85 14.58 14.32 14.42 14.62 14.62 14.71 14.53 14.27 14.94 14.55 14.55 14.48 14.42 14.42 14.24 14.24 14.25 14.43	3.12 3.09 3.19 3.18 3.21 3.20 3.15 3.16 3.13 3.06 3.12 3.17 3.17 3.13 3.14 3.14 3.12 3.14 3.13 3.14 3.13 3.11 3.11 3.11	

11663	-22.13	59.05	14.96	8.15	7.25	-18.53	46.15	14.74	14.16	3.26	
11664	-20.89	51.49	13.64	12.47	4.13	-19.02	46.48	13.90	14.60	3.18	
11665	-19.71	50.86	14.19	12.83	3.96	-18.34	46.39	14.60	14.67	3.16	
11666	-27.39	48.08	9.48	13.66	3.52	-26.62	45.34	9.67	14.27	3.18	
11667	-24.45	47.79	11.48	14.20	3.36	-23.94	43.95	11.78	14.07	3.12	
11671	-20.09	50.05	16.50	12.51	4.00	-18.56	45.44	16.67	14.52	3.13	
11672	-23.86	47.28	12.58	13.70	3.45	-23.25	45.35	12.93	14.55	3.12	
11672 B	-23.92	46.53	12.70	13.54	3.44						
11672 C	-23.91	46.97	12.72	13.63	3.45						
11673	-19.63	47.59	14.45	14.23	3.35	-19.22	45.05	14.79	14.48	3.11	
11674	-20.04	49.09	12.53	13.32	3.69	-19.03	45.17	12.81	14.29	3.16	
11675	-20.98	48.21	12.39	13.61	3.54	-20.31	45.16	12.65	14.24	3.17	
11677	-23.52	49.05	13.19	14.18	3.46	-22.86	46.09	13.40	14.73	3.13	
11678	-21.19	51.76	14.00	11.00	4.71	-18.84	45.99	14.20	14.54	3.16	
11678 B						-18.80	45.85	14.37	14.42	3.18	
11678 C						-18.82	45.91	14.28	14.43	3.18	
11802	-20.18	48.99	13.71	13.15	3.73	-19.27	45.44	14.13	14.14	3.21	
11805	-21.43	51.92	14.05	11.39	4.56	-19.44	45.85	14.39	13.76	3.33	
11807	-22.05	52.52	14.75	10.30	5.10	-19.49	46.62	15.09	14.00	3.33	
11811	-23.20	44.93	13.92	13.69	3.28	-23.12	43.83	14.18	14.02	3.13	
11812	-21.45	47.81	14.22	13.19	3.62	-20.56	44.21	14.55	14.04	3.15	
11812 B						-20.64	44.86	14.60	14.30	3.14	
11812 C						-20.68	44.72	14.59	14.20	3.15	
11814	-21.42	48.43	11.30	13.00	3.72	-20.69	44.80	11.50	14.12	3.17	
11820	-20.62	46.34	14.47	13.88	3.34	-20.38	43.57	14.65	13.93	3.13	
11821	-22.50	47.56	11.74	13.30	3.58	-21.96	45.01	12.12	14.24	3.16	
11823	-22.25	48.36	13.65	12.61	3.83	-21.14	44.82	13.90	14.03	3.19	
11826	-21.81	50.06	13.06	12.87	3.89	-20.76	44.70	13.38	14.16	3.16	
11830	-20.20	48.04	14.15	13.34	3.60	-19.42	44.72	14.35	14.18	3.15	
11831	-23.26	54.66	14.31	8.67	6.31	-19.62	46.61	14.67	14.29	3.26	
11831 B	-23.06	54.38	14.28	8.95	6.08						
11831 C	-23.23	55.06	14.35	8.73	6.31						
11832	-21.13	51.02	13.85	12.24	4.17	-19.53	45.97	14.19	14.26	3.22	
11834	-21.23	50.62	13.41	11.84	4.27	-19.47	45.49	13.82	14.23	3.20	
11835	-20.89	49.69	13.78	12.69	3.91	-19.46	45.89	14.07	14.49	3.17	
11836	-20.78	49.58	14.42	12.48	3.97	-19.42	45.55	14.55	14.22	3.20	
11837	-20.68	47.65	13.11	12.99	3.67	-21.06	44.93	14.45	14.22	3.16	
11837 B						-20.95	44.49	14.35	14.21	3.13	
11837 C	04.44	40.04	44.00	40.04		-20.95	41.53	14.28	13.26	3.13	
11839	-21.11	49.34	14.38	12.64	3.90	-19.52	45.71	14.55	14.19	3.22	
11841	-20.84	49.79	13.52	12.43	4.00	-19.25	45.51	13.71	14.31	3.18	
11040	-23.58	40.20	13.37	13.54	3.42	-23.07	44.03	13.03	14.29	3.14	
11047	-23.09	40.00	13.52	13.51	3.45	-22.09	44.40	13.91	14.23	3.12	
11040	-21.14	40.00	13.00	12.01	3.20	-21.00	43.33	14.23	13.00	3.13	
11000	-21.33	49.32	12.92	10.01	2.19	-20.10	45.07	14.10	14.24	0.14 0.10	
11000 11000	-22.07	49.07	12.93	12.34	3.90	-20.57	45.50	14.10	14.24	3.10	
11055 D	-22.20	49.42	13.92	12.09	4.09						
11055 C	-22.03	40.97 40 EE	14.06	12.07	2 5 5 5	21.07	12 CE	15 12	10 OE	2 1 5	
11957	-21.34	40.00	12 11	12.00	3.00	-21.07	43.03	12.13	14.26	3.10	
11850	-13.00	47.40	12.51	12.00	3.44	-15.05	44.55	12 70	12.52	3.12	
11860	-22.07	50.49	14.25	12.09	4.01	-21.31	42.55	14 77	1/ 22	3.14	
11862	-20.90	53.54	14.23	11 33	4.01	-19.40	45.00	15 1/	14.33	3.15	
11862 B	-21.45	55.54	14.75	11.55	4.75	-10.13	46.11	15 11	1/ 30	3.27	
11862 C						-10.24	45.58	15.13	1/ 13	3.22	
11865	-21.66	52 35	1/ 70	11 72	1 17	-10.12	46.27	15.13	1/ 00	3.22	
11867	-21.00	50 35	14.01	12.08	3.88	-10.53	45.46	13.65	1/ 17	3.20	
11868	-20.45	48 85	13.63	12.00	3 77	-10.37	45 / 12	13.05	14 11	3.22	
11871	-20.41	10.00	14 61	12.34	3 00	-10.00	/3 52	15.00	13.50	3.22	
11872	-21 26	46 84	14.54	13 72	3.41	-20.10	44 69	14.69	14.06	3.18	
11875	-20.63	46 18	8 54	13.63	3 39	-20.32	43.86	8.91	13.83	3.17	
11876	-22.90	47.46	11.70	13.58	3.49	-20.32	45.15	11.89	14.43	3,13	
11876 B	-22.91	47.50	11.67	13.44	3.53						
11876 C	-22.84	47.68	11.66	13.63	3,50						
11877	-21.95	52.19	15.17	9,58	5.45	-19.17	45,41	15.30	14,00	3.24	
11878	-20.55	51 11	13 50	13 02	3,93	-19.24	45.67	14.07	14,25	3.21	
11879	-20.41	48.73	13.49	13.33	3.66	-19.49	45.75	14.21	14.24	3,21	
11883	-20.97	46.59	14.65	13.96	3.34	-20.73	44.21	15.00	14.05	3.15	
11886	-21.07	45.11	15.03	13.45	3.35	-20.29	57.50	15.29	18.50	3.11	
11886 B						-20.45	61.02	15.29	19.60	3.11	
11886 C						-20.28	43.16	15.35	13.93	3.10	
11887	-21.26	46.53	13.14	13.43	3.46	-20.75	41.14	13.36	13.45	3.06	
11888	-21.44	46.64	14.17	13.84	3.37						
11889	-22.46	49.79	14.44	11.76	4.23	-20.23	44.56	14.80	14.39	3.10	
11894	-19.59	46.42	15.30	13.76	3.37	-19.27	45.42	15.84	14.34	3.17	
11895	-23.23	51.14	13.20	11.20	4.56	-20.92	44.40	12.95	14.46	3.07	
11896	-23.24	50.65	14.14	11.26	4.50	-21.23	44.96	14.53	14.17	3.17	
11899	-20.99	48.09	14.49	13.64	3.53	-20.52	43.72	14.86	13.93	3.14	
11899 B	-20.92	48.68	14.66	14.00	3.48						
11899 C	-20.96	48.42	14.68	13.88	3.49						
11900	-22.65	49.27	13.78	12.66	3.89	-21.27	43.50	14.29	13.88	3.13	
11902	-19.46	46.62	13.51	13.66	3.41	-18.81	54.93	13.78	17.22	3.19	
11903	-20.27	49.31	13.62	13.47	3.66	-19.26	44.65	13.72	14.23	3.14	
11906	-19.48	33.70	14.55	9.87	3.41	-19.37	38.85	15.06	12.25	3.17	
11907	-20.51	46.83	14.28	13.87	3.38	-19.75	42.80	14.17	13.92	3.07	
11009	-20.02	15 25	1/ /0	13 38	3 38	-20.69	41 72	14.62	13 31	3 13	

11908 B						-20.41	42.90	14.53	13.73	3.12	
11908 C						-20.25	42.05	14.71	13.23	3.18	
11916	-22.56	46.80	13.18	13.91	3.36	-22.19	31.43	13.40	9.74	3.23	
11918	-24.77	48.51	11.07	13.54	3.58	-24.00	45.03	11.08	14.06	3.20	
11920	-23.50	47.08	12.73	13.24	3.56	-22.91	45.08	12.97	13.99	3.22	
11921	-22.72	44.82	13.75	12.01	3.73	-21.57	42.68	13.39	13.60	3.14	
11923	-20.20	46.46	16.15	13.84	3.36	-19.86	43.07	16.18	13.54	3.18	
11924	-23.63	45.67	12.54	14.02	3.26	-23.44	43.56	12.65	13.73	3.17	
11924 B	-23.59	45.40	12.47	13.95	3.26						
11924 C	-23.63	45.45	12.28	14.02	3.24						
11927	-22.64	46.70	10.81	13.62	3.43	-22.25	40.23	11.05	12.50	3.22	
11929	-23.28	47.06	11.43	13.64	3.45	-22.83	45.68	11.98	14.04	3.25	
11930	-23.06	46.77	11.34	13.16	3.55	-22.57	45.56	11.80	14.01	3.25	
11932	-20.24	48.20	13.98	13.09	3.68	-19.21	46.78	14.66	14.40	3.25	

## Appendix H Raw data: fatty acid analysis

Muscle tissue fatty acid proportions (% total fatty acids) of Arctic char individuals (ID) from Qasigiyat.

	Fatty Acid																					
ID	C14:0	C16:0	C16:1n7	C18:0	C18:1n11	C18:1n9	C18:1n7	C18:1n5	C18:2n6	C18:3n3	C18:4n3	C20:1n11	C20:1n9	C20:1n7	C20:3n6	C20:4n6	C20:4n3	C20:5n3	C22:1n11	C22:1n9	C22:5n3	C22:6n3
805	2.644	12.577	10.044	1.468	1.229	10.634	2.729	0.647	0.803	0.299	1.446	1.306	8.215	1.090	0.137	0.468	1.011	13.242	7.420	1.756	3.023	12.724
807	3.275	11.888	11.616	1.688	0.972	12.398	3.219	0.681	0.946	0.353	1.767	1.159	7.509	0.995	0.168	0.439	1.160	11.632	6.027	1.460	3.116	11.889
814	1.951	17.504	10.050	3.543	0.220	10.599	4.606	0.257	8.745	2.004	0.325	0.056	0.832	0.214	0.606	2.620	0.544	7.061	0.086	0.120	2.271	16.989
821	1.915	17.583	9.388	3.889	0.396	10.326	4.592	0.273	8.359	1.808	0.322	0.083	0.931	0.221	0.502	2.699	0.502	7.014	0.082	0.115	2.555	18.474
831	4.427	12.036	11.894	1.639	1.061	12.177	3.248	0.775	0.851	0.349	1.747	1.340	8.598	1.074	0.175	0.346	1.142	10.981	7.601	1.571	2.723	7.905
835	3.083	13.178	9.849	1.761	0.983	10.650	2.794	0.712	0.771	0.347	1.546	1.076	7.558	1.000	0.152	0.538	1.079	13.497	6.974	1.652	2.869	12.402
846	0.637	17.735	3.193	4.784	0.301	6.436	2.596	0.181	3.937	1.417	0.218	0.051	0.527	0.122	0.365	3.767	0.459	8.432	0.329	0.126	3.062	36.168
847	0.565	17.563	2.529	4.392	0.432	7.019	2.760	0.223	2.513	0.569	0.121	0.088	0.817	0.177	0.271	3.255	0.455	8.207	0.078	0.149	4.163	39.050
848	0.599	18.046	1.636	3.860	0.523	5.101	2.240	0.579	0.341	0.141	0.291	0.107	1.046	0.159	0.114	1.357	0.550	15.698	0.243	0.108	3.098	40.278
853	2.578	14.238	8.504	2.232	0.846	7.534	2.579	0.991	0.601	0.278	1.504	0.594	5.430	0.763	0.215	0.548	0.907	15.972	5.449	1.211	3.402	17.417
855	2.974	14.267	9.095	2.278	0.894	9.638	3.457	1.045	0.707	0.313	1.275	0.565	5.281	0.642	0.149	0.504	0.963	14.149	4.915	1.042	3.308	16.355
856	1.107	16.589	6.246	3.567	0.656	13.120	4.183	0.399	2.097	0.844	0.291	0.158	1.971	0.334	0.275	1.472	0.781	7.047	0.379	0.287	4.258	29.713
857	1.026	15.424	5.305	3.344	0.594	13.976	3.678	0.354	3.071	1.793	0.397	0.164	1.812	0.308	1.809	1.958	0.836	6.422	0.295	0.274	3.664	28.724
862	3.020	13.593	11.394	2.319	1.089	16.413	3.746	0.637	1.000	0.295	1.084	1.213	7.041	0.076	0.207	0.439	0.981	10.056	4.411	1.066	2.908	12.100
865	3.406	12.810	10.656	2.079	0.907	12.875	2.905	0.617	0.710	0.287	1.659	1.115	7.334	0.941	0.174	0.426	1.099	11.541	6.599	1.447	2.647	11.840
868	2.590	15.038	9.773	2.185	0.762	11.547	3.207	0.583	0.756	0.347	1.350	0.615	4.244	0.640	0.179	0.641	1.186	15.795	3.444	0.879	4.031	15.178
871	2.425	15.373	7.927	3.370	0.710	13.857	4.123	1.104	0.877	0.351	0.881	0.251	3.062	0.385	0.191	0.630	0.902	13.511	1.885	0.500	3.316	18.695
872	1.270	19.151	5.678	3.611	0.117	12.284	3.301	0.278	3.571	1.306	0.362	0.060	0.879	0.170	2.840	2.840	0.638	7.935	0.065	0.106	2.192	28.298
875	1.004	18.823	2.978	3.781	0.095	6.531	2.347	0.135	3.858	1.735	0.425	0.015	0.316	0.094	0.773	6.549	0.829	10.125	0.071	0.072	2.233	30.198
876	1.405	17.574	8.110	3.397	0.255	10.014	4.185	0.265	6.931	2.089	0.541	0.068	0.807	0.194	0.611	2.564	0.761	6.098	0.083	0.129	3.019	23.766
878	2.811	14.066	10.494	1.980	0.988	12.641	3.026	0.632	0.779	0.300	1.375	1.165	6.768	0.848	0.093	0.504	1.083	13.108	5.272	1.246	2.813	12.989
886	0.441	16.985	1.594	5.973	0.123	8.452	3.086	0.266	1.272	0.393	0.101	0.033	0.566	0.145	0.195	4.210	0.314	8.421	0.135	0.092	2.386	39.847
894	0.589	19.395	3.094	4.278	0.263	8.499	2.843	0.245	1.729	0.773	0.125	0.052	0.723	0.145	1.410	2.502	0.511	7.881	0.072	0.135	2.719	36.775
895	2.670	15.597	8.498	2.668	0.712	14.988	3.620	1.016	1.058	0.506	0.952	0.350	3.952	0.430	0.206	0.592	0.872	12.761	3.410	0.783	2.872	15.682
896	2.668	15.350	8.697	3.348	0.622	15.779	3.754	1.087	1.006	0.367	0.859	0.384	3.982	0.471	0.176	0.630	0.805	11.694	3.015	0.738	2.983	16.080
899	1.257	18.305	5.272	4.362	0.094	15.002	3.521	0.266	3.438	1.259	0.234	0.027	0.838	0.158	0.307	2.481	0.556	7.002	0.046	0.125	2.369	27.627
900	2.400	14.816	7.783	3.535	0.674	13.727	3.830	1.256	0.841	0.331	0.897	0.368	4.061	0.572	0.234	0.628	0.891	12.664	3.402	0.843	3.525	16.917
906	0.868	19.948	2.785	6.126	0.201	8.740	3.166	0.284	1.867	0.849	0.174	0.037	0.518	0.148	0.227	2.694	0.549	7.147	0.059	0.097	2.725	35.123
908	0.658	18.206	2.368	5.800	0.146	7.138	2.882	0.199	2.710	1.073	0.145	0.040	0.481	0.138	0.316	3.869	0.489	7.647	0.023	0.097	2.788	36.882
916	0.922	16.970	4.617	4.369	0.470	11.399	3.418	0.328	2.362	0.695	0.196	0.144	1.705	0.267	0.315	2.890	0.583	9.228	0.271	0.232	3.788	29.805
921	1.468	16.476	7.789	3.153	0.696	13.747	4.089	0.396	3.790	0.886	0.456	0.331	2.541	0.398	0.308	1.956	0.755	8.021	1.128	0.387	3.363	22.525
927	1.618	18.414	7.527	3.988	0.102	8.591	3.880	0.222	8.005	2.270	0.367	0.046	0.545	0.163	0.671	3.704	0.423	9.099	0.176	0.104	2.242	18.979

											Fat	ty Acid										
ID	C14:0	C16:0	C16:1n7	C18:0	C18:1n11	C18:1n9	C18:1n7	C18:1n5	C18:2n6	C18:3n3	C18:4n3	C20:1n11	C20:1n9	C20:1n7	C20:3n6	C20:4n6	C20:4n3	C20:5n3	C22:1n11	C22:1n9	C22:5n3	C22:6n3
602	1.100	18.570	5.560	4.720	0.160	8.860	4.450	0.210	5.950	2.170	0.980	0.060	0.520	0.180	0.500	4.800	1.030	8.470	0.530	0.170	2.630	21.290
604	1.510	17.340	6.210	4.500	0.070	9.260	4.570	0.320	8.440	2.720	0.980	0.020	0.520	0.170	0.590	3.940	0.870	9.550	0.080	0.100	2.180	16.760
607	2.980	15.680	8.500	2.610	0.570	9.580	3.880	0.460	0.780	0.410	1.230	0.380	3.470	0.520	0.160	1.040	0.980	15.730	2.970	0.720	3.210	17.750
608	1.690	17.520	4.770	3.150	0.660	9.350	3.560	0.490	0.480	0.200	0.490	0.310	4.020	0.480	0.120	0.910	0.610	12.490	3.050	0.680	3.110	26.630
609	3.860	14.990	10.650	2.460	0.460	11.440	3.800	0.430	0.760	0.300	1.450	0.340	4.270	0.550	0.150	0.580	0.920	14.560	2.980	0.680	2.800	15.110
612	3.380	15.510	8.970	2.830	0.490	18.390	3.380	0.610	0.590	0.210	0.380	0.470	7.340	0.710	0.080	0.500	0.470	7.850	4.690	1.050	2.620	14.710
613	3.690	15.830	11.990	2.880	0.480	17.950	4.530	0.590	0.850	0.330	0.820	0.420	5.090	0.680	0.120	0.550	0.850	9.240	2.940	0.720	3.210	10.960
615	1.440	18.660	7.620	4.190	0.080	10.550	4.330	0.220	6.530	3.790	2.140	0.050	0.480	0.130	0.450	3.360	1.580	9.480	0.120	0.090	2.570	15.250
616	3.510	15.120	8.460	3.120	0.740	13.760	3.590	0.630	0.530	0.210	0.760	0.550	7.960	0.820	0.100	0.450	0.610	9.580	6.050	1.260	0.020	13.580
617	1.620	16.160	8.800	4.790	0.070	11.120	5.720	0.230	7.320	3.400	1.410	0.080	0.620	0.210	0.660	3.980	1.400	6.700	0.130	0.110	0.020	14.670
618	2.130	16.580	9.960	4.590	0.160	11.980	4.940	0.300	7.730	3.310	1.420	0.120	1.160	0.260	0.520	2.620	1.070	8.450	0.010	0.170	2.370	11.450
621	4.440	14.550	18.660	2.460	0.090	16.270	4.600	0.290	1.850	0.420	1.390	0.250	1.880	0.530	0.230	0.510	1.200	11.270	0.640	0.280	3.990	8.390
625	4.210	16.720	11.210	2.660	0.070	9.560	3.470	0.280	1.040	0.450	1.830	0.070	0.890	0.310	0.210	0.820	1.260	17.430	0.260	0.160	3.200	16.800
628	1.520	18.480	4.680	4.500	0.160	13.750	3.850	0.640	0.470	0.320	0.670	0.070	1.230	0.220	0.100	0.520	0.750	13.180	0.420	0.220	2.870	26.060
632	0.640	22.620	0.070	4.060	0.090	5.290	2.820	0.170	2.880	1.140	0.290	0.020	0.370	0.220	0.540	9.640	0.770	7.700	0.180	0.070	4.170	28.350
633	3.050	13.190	14.790	2.540	0.140	18.250	4.120	0.430	1.530	0.340	1.020	0.240	1.950	0.490	0.210	0.640	0.930	11.830	0.720	0.320	3.910	13.760
635	1.480	16.770	8.670	4.630	0.120	10.260	4.550	0.230	7.480	2.330	1.000	0.030	0.470	0.130	0.680	4.570	0.780	11.100	0.040	0.090	2.330	13.660
637	0.930	17.950	7.150	4.630	0.090	10.200	4.790	0.190	7.250	3.370	1.190	0.040	0.340	0.150	0.360	4.650	1.090	7.840	0.080	0.090	2.210	18.110
638	0.720	20.820	3.930	3.760	0.100	6.460	3.150	0.180	3.270	1.110	0.350	0.050	0.450	0.170	0.470	8.390	0.630	7.540	0.190	0.090	2.820	27.890
639	3.310	10.960	13.510	1.970	0.830	17.270	3.800	0.490	1.120	0.300	1.010	0.860	9.050	0.930	0.190	0.410	0.980	9.220	4.900	1.140	3.060	9.090
641	3.610	14.400	17.680	2.190	0.280	16.920	4.440	0.340	1.380	0.370	1.270	0.400	3.350	0.580	0.190	0.450	1.080	10.850	1.530	0.450	3.400	9.160
643	2.220	16.300	6.760	3.650	0.200	15.630	4.150	0.600	0.600	0.380	1.110	0.200	2.390	0.400	0.100	0.470	0.900	12.680	1.700	0.540	2.850	20.500
644	1.800	16.550	4.870	3.400	0.330	10.710	3.410	0.600	0.610	0.330	0.780	0.170	2.530	0.320	0.100	0.700	0.740	13.310	1.530	0.410	2.910	27.470
648	2.310	16.300	6.490	2.840	0.360	11.470	3.730	0.510	0.600	0.330	0.840	0.230	3.100	0.440	0.120	0.760	0.750	13.220	2.260	0.550	2.940	23.620
650	2.700	15.670	7.630	3.220	0.330	9.950	3.530	0.490	0.790	0.400	1.200	0.170	1.950	0.390	0.150	0.740	0.940	14.300	1.280	0.380	3.240	23.690
660	1.760	16.750	5.180	3.400	0.220	9.160	3.770	0.530	0.820	0.470	0.830	0.130	1.390	0.290	0.070	1.150	1.030	14.530	0.720	0.240	3.240	28.210
662	1.160	17.050	6.490	4.520	0.230	12.170	5.110	0.270	4.910	1.690	0.850	0.040	0.980	0.220	0.380	3.150	0.880	7.760	0.070	0.140	3.630	22.380
663	3.920	11.500	15.390	2.420	0.600	19.030	3.840	0.400	1.440	0.360	1.200	0.620	6.310	0.760	0.190	0.360	1.110	9.530	3.410	0.880	3.130	7.690
665	2.620	14.300	14.550	2.360	0.700	13.580	4.320	0.430	0.900	0.320	0.900	0.640	6.040	0.800	0.150	0.500	0.850	11.450	3.900	0.910	3.340	10.940
666	1.360	17.810	6.870	3.640	0.130	10.950	3.520	0.170	3.680	3.040	2.840	0.030	0.460	0.100	0.420	2.930	2.090	9.160	0.030	0.080	2.400	21.440
667	0.750	17.680	4.070	4.580	0.230	10.490	3.920	0.220	3.910	1.660	0.750	0.050	0.780	0.170	0.510	3.630	1.130	8.400	0.050	0.100	3.160	28.090
677	0.760	20.080	4.640	4.180	0.080	7.800	3.960	0.220	3.420	1.170	0.360	0.050	0.430	0.170	0.170	6.910	0.660	7.200	0.060	0.070	4.170	25.360
678	3.400	13.640	15.030	2.780	0.210	18.010	4.440	0.390	1.610	0.340	1.060	0.270	2.510	0.510	0.200	0.560	0.990	11.400	1.120	0.360	3.830	11.660

Muscle tissue fatty acid proportions (% total fatty acids) of Arctic char individuals (ID) from Iqalugaarjuit.