SIZE-AT-AGE AND DIET COMPOSITION OF PACIFIC HALIBUT (*HIPPOGLOSSUS STENOLEPIS*) IN COOK INLET, ALASKA

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ABSTRACT

SIZE-AT-AGE AND DIET COMPOSITION OF PACIFIC HALIBUT (*HIPPOGLOSSUS STENOLEPIS)* IN COOK INLET, ALASKA.

Since the 1970s halibut size-at-age has decreased in southcentral Alaska; the mechanisms causing decreased size-at-age are unknown. The objectives of this study were to 1) compare size-at-age of port-sampled fish in Homer to survey samples from Gulf of Alaska; 2) assess stable isotope values ($\delta^{13}C$, $\delta^{15}N$) of halibut by sex, size, location and date; 3) determine mean stable isotope values for prey; and 4) identify prey associated with smaller and larger size-at-age. We used port-sampled halibut from the Homer sport fishery due to the quantity of available carcasses. Port-sampled fish were generally larger than survey sampled fish from the same region. Halibut had a wide range of stable isotope values that varied with all factors. Prey isotope values were wide and overlapping, allowing for distinctions among teleost, cephalopods, crustaceans and amphipods. Older and younger fish of the same size and sex had different proportions of prey assimilated into their muscle.

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GENERAL INTRODUCTION

Understanding the relationships between individual fish size-at-age, growth and population biomass is critical for management of exploited fish stocks (Beverton and Holt 1993). Generally, fish growth is governed by bioenergetic processes including consumption, respiration, heat increment of feeding, excretion and egestion (Kitchell *et al*. 1977, Wootton 1999, Helfman *et al.* 2009), and growth models are used to project the number of fish by age into the future. Ultimately fish stock biomass is determined by the number of fish and their size (weight)-at-age. In fisheries where implicit or explicit size or age-based rules (i.e. mesh size or minimum fish size limits) are used to manage harvest, changes in fish growth directly impact the "exploitable" portion of the stock. Over the past several decades, Pacific halibut (*Hippoglossus stenolepis*) size-at-age has declined substantially, resulting in decreased exploitable biomass under the current 81cm minimum fork length commercial harvest restriction (IPHC 2013b). The mechanisms causing decreased halibut growth are unknown.

Pacific halibut are right-eyed flatfish (order Pleuronectiformes, family Pleuronectidae; Mecklenburg *et al.* 2002) distributed along the continental shelves of the North Pacific Ocean and Bering Sea from Hokaido, Japan, to Northern California, USA, with their highest concentrations centered around Kodiak Island, Alaska (IPHC 2011). Mature halibut congregate off the continental shelf to spawn between November and March. Females are highly fecund, releasing 500,000 to 4 million eggs annually (Bell 1981). Eggs are free floating and hatch after approximately 15 days. Larvae and post-larvae are pelagic and oceanographic processes determine their distributions. Halibut remain in the post-larval stage for approximately six months, before settling on the continental shelf and taking on their adult form (Bell 1981).

Females grow faster, but mature later than males and sexual maturity occurs at 12 and 8 years, respectively. Halibut may live up to 55 years, be up to 2.5 m long and weigh up to 230 kg (IPHC 1998).

Halibut are culturally, economically and ecologically important in the North Pacific. Native communities have harvested halibut for thousands of years and commercial long-line fisheries in Alaska and British Columbia have been active since 1888 (IPHC 2008, 2011). In 2012, halibut ranked 11th of all species for total value of fish landed in the United States and 5th for the state of Alaska. Ex-vessel value in Alaskan waters was estimated to be $144.8 million (NMFS 2012). Southcentral Alaskan waters (IPHC management area 3A; Figure 1) have substantial commercial, sport and subsistence halibut fisheries. The ex-vessel value of the commercial fishery in 3A in 2012 was approximately $68.3million (IPHC 2013b, National Marine Fisheries Service 2013) and accounted for the largest proportion of all commercially caught halibut (IPHC 2013b). In 2013, combined landings of Pacific halibut in 3A accounted for 52.8% of the total landings from all Alaskan halibut fisheries (IPHC 2013b). In 2013, sport fishery landings in 3A (consisting of both charters and private fishers) were about 3.7 million pounds, accounting for 69% of sport caught halibut in Alaska (IPHC 2014). Homer is the top halibut sport fishing port in Alaska with an estimated 72,636 fish harvested in 2013 (Meyer and Powers 2013).

The International Pacific Halibut Commission (IPHC) is a cooperative government agency between the United States and Canada which studies and manages Pacific halibut. The IPHC was created in 1923, when the Convention for the Preservation of the Halibut Fishery of the Northern Pacific Ocean including the Bering Sea was signed by both countries (Clark *et al.* 1999).

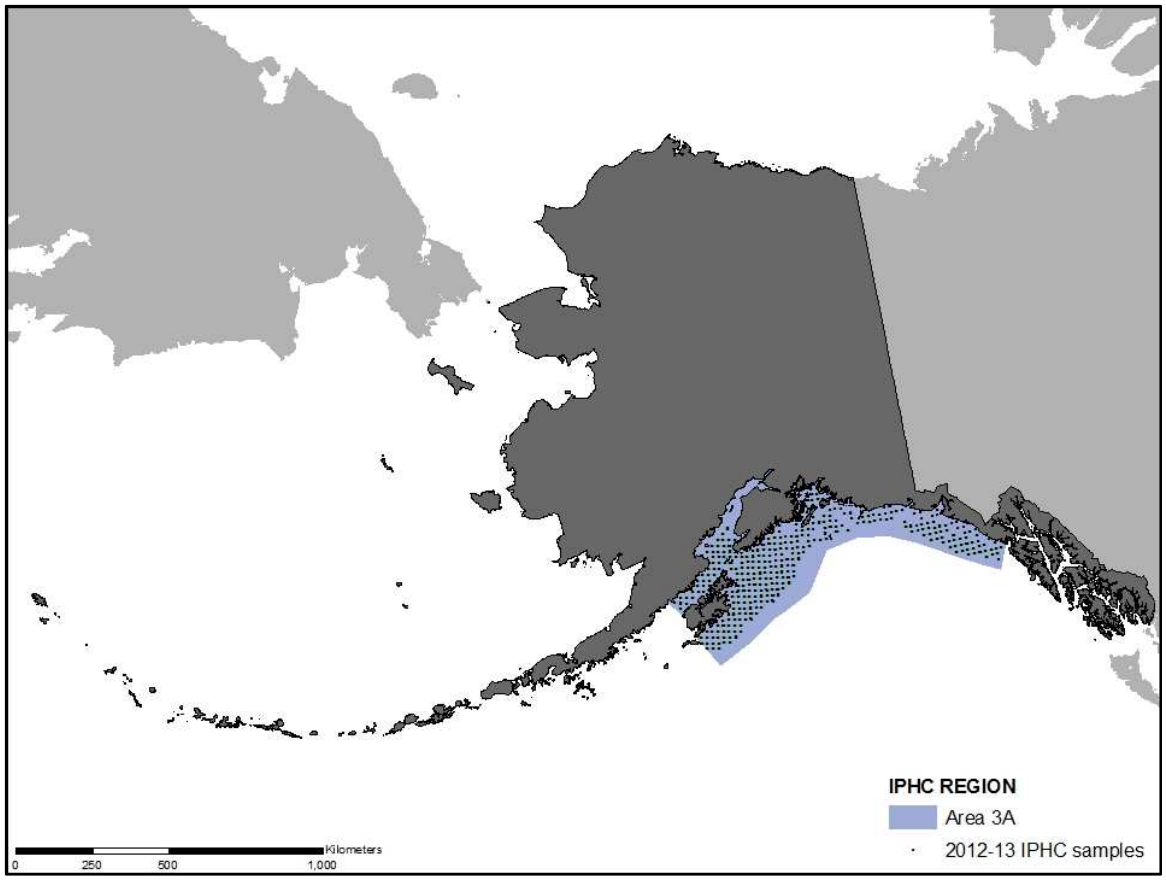

Figure 1. International Pacific Halibut Commission regulatory area 3A; points are all of the sample locations for the 2014 annual stock assessment survey (IPHC 2014).

Initially, this international agreement was economically motivated and called for a winter closure of the fishery. The Royal Commission in Canada also placed an emphasis on the importance of conservation in light of the already declining Pacific halibut stock (Bell 1981). At the time, the convention called for an investigation of the life history of halibut with recommendations for regulations for the preservation and development of the fishery (Bell 1981). The ultimate goal of the commission has consistently been to develop and maintain the halibut stocks at levels that would provide maximum sustainable yield (Bell 1981). This requires an understanding of the mortality, recruitment and growth characteristics of the managed species to ensure that harvest levels do not exceed stock growth (Jennings *et al.* 2001).

Since the inception of the IPHC, management of the halibut fishery has relied on varying methods for stock assessment and harvest policy approaches (IPHC 2011). Annually, the IPHC conducts both setline and trawl surveys to estimate the total and exploitable halibut biomass. Stock assessment surveys require simultaneous sampling on many vessels; for example, the 2012 survey required 10 vessels with a total of 686 charter days (IPHC 2013d). Survey long-line sets are made in United States and Canadian waters from Oregon to the Bering Sea (total 1,274 stations in 2012) on a 10x10 nautical mile grid in depths ranging from 35 to 500 m (see Figure 1). This information is pooled with information from the commercial fishery catch and discards along with sport fishery harvest to determine the next year's harvest and size limitations for both commercial and sport fisheries (IPHC 2013b). In Alaska, the Alaska Department of Fish and Game (ADFG) is responsible for collecting information on sport caught halibut through port sampling, interviews and charter logbooks (IPHC 2013b).

Despite steady or increasing halibut biomass in area 3A over the past two decades, mean fish sizes-at-age have been declining (IPHC 2006). The IPHC reports that mean sizes-at-age for both males and females increased from the 1920s – 1970s, then began to decline, and are again near 1920 levels (IPHC 2013b). The exact timing of the decline is not well documented and may have begun in the late 1970s (Clark *et al.* 1999) or in the early 1980s (Clark and Hare 2002). The result is a shrinking proportion of fish in the exploitable size range (≥81 cm), leading to reduced harvest allocations even when fish abundance is high. Setline survey data are used in assessing size-at-age trends (IPHC 2006); however, there are several instances where gaps in sampling or changes in protocol may confound size-at-age information (IPHC 2001, 2013a).

Here, I examine halibut size-at-age in relation to diet using port-sampled fish from Homer, Alaska. In chapter 1, I explore the growth characteristics of port-sampled versus IPHC sampled fish. The null hypothesis was that there were no differences in size-at-age between these data sets. In all but one case, the null hypothesis was rejected; port-sampled fish were significantly larger than those collected on IPHC surveys both regionally (regulatory area 3A) and locally (statistical area 261).

In the sport and commercial fisheries, size-at-age is measured using fork length, or the distance from the snout to the center of the caudal fin (IPHC 2013b). Fork length is translated to weight-at-age using an allometric equation to allow stock biomass assessments because the halibut fishery landings are recorded as weight, not numbers of fish. While the IPHC and ADFG annual surveys measure size-at-age of many halibut, the actual drivers of growth have rarely been investigated because they are difficult and expensive to assess.

Potential mechanisms influencing halibut growth include changes in abundance of halibut, recruitment, fishing pressure, climate, prey availability, disease and abundance of other species. Clark *et al*. (1999) suggested that a Pacific Decadal Oscillation (PDO) climate regime shift, starting in 1976-77, resulted in increased water temperatures, and drove increases in halibut abundance. Clark and Hare (2002) refuted the correlation to the PDO and agreed that increased abundance of halibut might be causing a decrease in growth rate via competition for food. The growth rates of Pacific halibut vary with depth, geographical location and gender (Orlov *et al*. 2011); forage varies in relation to each of these factors and may be the driver of growth differences (IPHC 2008). Reduced growth rates of individual fish may be the result of declines in prey availability or quality due to environmental changes, or competition with other species (e.g. Arrowtooth flounder, *Atheresthes stomias*) (IPHC 2013b). Selective fishing mortality was also proposed as a mechanism for decreased size-at-age for halibut and is documented in other teleosts with minimum size limits (Conover and Munch 2002, Stephen *et al.* 2011, IPHC 2013b). Disproportionate targeting of large fish over extended periods can result in the evolution of slower growing fish (Lee 1912, IPHC 2013b). Gaichas *et al.* (2011) suggest that fishing pressure and predator-prey dynamics have the greatest impacts on halibut biomass. Finally, the IPHC and USGS have recently documented the fish parasite *Ichthyophonus hoferi* in halibut (IPHC 2013c), which causes decreased growth in other teleost fishes (Kocan *et al.* 2010).

While numerous factors have been implicated in this issue, in chapter 2 I focus on one of those factors, ingested energy. Halibut diets have been described only generally using gut content analyses. Known prey include fishes, crustaceans, mollusks, echinoderms, cephalopods, marine

worms, kelp and fisheries offal (IPHC 1986, 2000, 2008, Orlov and Moukhametov 2007, Roseneau and Byrd 2000). The 1999 and 2001 Alaska Fisheries Science Center (AFSC) surveys of commercially important groundfishes in the Gulf of Alaska found that walleye pollock (*Theragra chalcogramma*), Pacific sand lance (*Ammodytes hexapterus*) and capelin (*Mallotus villosus*) contributed the most, by weight, to halibut diet. Hermit crabs (*Pagurus sp.)*, by weight of invertebrate species, were also a top contributor (NOAA 2006A). In Cook Inlet, juvenile and adult halibut eat capelin, sand lance, flatfish, sculpin (*Cottidae, sp.)*, Pacific cod (*Gadus macrocephalus)*, crabs, shrimp, squid, octopuses, and mollusks (Roseneau and Byrd 2000). Anecdotal information from fishers in the Port of Homer suggests that halibut are now consuming more crustaceans (especially crabs) and fewer forage fish.

Stomach content analyses can provide definitive species-level prey samples but are of limited use for diet-growth studies. Stomach contents only provide a recent "snapshot" of what the fish has consumed, and are often missing due to frequent regurgitation during capture. Further, these analyses provide a potentially biased perspective due to rapid (hrs to days) and variable prey digestion rates (Berens and Murie 2008). Crustacean exoskeletons, for example, may remain in the stomach for longer periods of time compared to food with more easily digestible parts, such as fish prey (Hopkins and Larson 1990).

Stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) have been used to successfully evaluate the diets of terrestrial and marine species including teleost fishes (for examples see Fry 1988, Benstead *et al.* 2006, Fernandez *et al.* 2011). Through this method, a range of proportional contribution of prey to diet is constructed (Fernandez *et al* 2011). When a fish consumes prey, its tissues retain the

stable isotope (e.g. $\delta^{13}C$ and $\delta^{15}N$) ratios of prey in proportion to assimilation (not consumption) (Phillips 2012, Marsh *et al.* 2012). However, assimilation rates (the rate at which prey is incorporated into the predator's tissues) and turnover rates (the time it takes for a given consumer tissue to reflect the isotopic composition of food resources; the result of both tissue growth and tissue replacement) vary between tissue types (Madigan *et al.* 2012). For example, analyzing both muscle and otolith tissue from the same fish provides information on dietary inputs at the scale of months and years, respectively (Carleton and Martinez del Rio 2005; Carleton *et al*. 2008; Martinez del Rio *et al.* 2009, Marsh *et al.* 2012). The IPHC (2003) and Marsh *et al.* (2012) used $\delta^{13}C$ and $\delta^{15}N$ to evaluate halibut trophic level, but no studies have applied stable isotope analyses to assess proportional dietary contributions of halibut prey.

In Chapter 2, I describe the diet of halibut based on samples from the sport fishery in Homer. The analysis focuses on describing the stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in halibut and their prey. Prey analysis was necessary to establish $\delta^{13}C$ and $\delta^{15}N$ values of prey before applying them to dietary analysis of halibut. The null hypothesis that there were no differences in the mean stable isotope values of halibut prey groups was rejected. I next examined the $\delta^{13}C$ and $\delta^{15}N$ ratios in halibut flesh and compared those by sex, sample date, location, size and age. I rejected the null hypotheses that there were no differences in $\delta^{13}C$ and $\delta^{15}N$ ratios among groups for all factors. Finally, I used Bayesian implementations of mixture models to establish a range of proportional contribution of prey to halibut diet relative to size-at-age.

This project relied heavily on partnerships with the Alaska Department of Fish and Game, Division of Sport Fish, Groundfish Research Program in Homer. The port-sampling program

provided assistance throughout the 2012 pilot season in forming relationships with fishermen and insight on supplies and field methods. The project also relied heavily on relationships with the charter industry and fillet businesses in the port of Homer, such as North Country Charters and Buttwhackers.

The field sampling for this project was done in collaboration with Caitlin Grenier's Master's thesis sampling. Caitlin studied *Ichthyophonus hoferi* prevalence in Cook Inlet halibut and examined parasite intensity in the heart. In addition to our individual thesis work, we will examine *Ichthyophonus* data relative to size, age and diet of fish. The current method of *Ichthyophonus* transmission is poorly understood (Kocan *et al.* 2010) and diet data could provide valuable information on routes of infection in halibut. *Ichthyophonus* is known to impair organ function in other fish and therefore could be impacting halibut growth (Kocan *et al.* 2010).

CHAPTER 1

PACIFIC HALIBUT SIZE-AT-AGE

1.1 INTRODUCTION

In 2012, commercial, sport and personal use landings of Pacific halibut (*Hippoglossus stenolepis*) in southcentral Alaska (International Pacific Halibut Commission (IPHC) regulatory area 3A) were estimated to account for 51.5% of the total landings from Alaskan halibut fisheries (IPHC 2013b). During the same year, the ex-vessel value of the commercial fishery in 3A was approximately $68.3million (IPHC 2013b, NMFS 2013). Since 1973, international regulation has prohibited commercial harvest of halibut less than 81 cm fork length (32 in); annual harvest levels are determined based on the biomass of fish in the "exploitable" size range (≥81 cm). Despite steady or increasing levels of halibut biomass in area 3A over the past two decades, fish size-at-age is declining (IPHC 2006a). The result is a shrinking proportion of fish in the exploitable size range, leading to reduced harvest allocations even when fish abundance is high. The causes of declining halibut size-at-age are unknown.

Annual growth of fishes is the result of many factors integrated over time and space such as temperature, food availability, nutrient availability, light regime, oxygen, salinity, pollutants, current speed, predator density, intraspecific social interactions and genetics (Helfman *et al*. 2009) and as such, Pacific halibut size-at-age varies both temporally and spatially (Clark *et al*. 1999, Clark and Hare 2002, Orlov *et al.* 2011, IPHC 2013b). Factors influencing halibut size-at-age include changes in abundance, recruitment, fishing pressure, climate, prey availability, disease and abundance of other species (Lee 1912, Clark *et al*. 1999, Clark and Hare 2002, Conover and Munch 2002, Gaichas *et al.* 2011, Orlov *et al*. 2011, Stephen *et al.* 2011, Kocan *et al*. 2010, IPHC 2013b). Clark and Hare (2002) hypothesized that increased abundance of halibut might be causing decreased size-at-age via competition for food. In addition, halibut forage

varies with depth, geographical location and gender (Orlov *et al*. 2011) and may be the driver of size-at-age differences (IPHC 2008). Reduced size-at-age may be the result of declines in prey availability or quality due to environmental changes, or competition with other species (e.g. Arrowtooth flounder, *Atheresthes stomias*) (IPHC 2013b). Selective fishing mortality has also been proposed as a mechanism for decreased halibut size-at-age and is documented in other teleosts with minimum size limits (Conover and Munch 2002, Stephen *et al.* 2011, IPHC 2013b). Gaichas *et al.* (2011) suggest that fishing pressure and predator-prey dynamics have the greatest impacts on halibut biomass. Finally, the IPHC, US Geological Survey (USGS) and Alaska Pacific University (APU) have recently documented the fish parasite *Ichthyophonus hoferi* in halibut (IPHC 2013a, APU FAST Lab unpublished data), which causes decreased growth in other teleost fishes (Kocan *et al*. 2010).

Presently, most fieldwork related to halibut ecology is done on stock assessment cruises. IPHC setline surveys encompass United States and Canadian nearshore and offshore waters from Oregon to the Bering Sea with regular stations (total 1,274 stations in 2012) on a 10x10 nautical mile grid at depths of 35 to 500 meters. The 2012 survey required 10 vessels with a total of 686 charter days (IPHC 2013d). Due to the cost and scope of projects that are included in the surveys, additional large-scale sampling to examine factors impacting size-at-age is not practical. Port sampling is used by the IPHC and other management agencies (e.g. Alaska Department of Fish and Game (ADFG)) to assess size-at-age in commercial and sport catches of halibut and other fishes (e.g. Scheirer *et al.* 2004, IPHC 2006a). In addition to standard sampling measures, ADFG used port sampling during 2011 and 2012 to assess *Ichthyophonus* and Mushy Flesh Syndrome (MFS) in halibut and other groundfish (APU FAST Lab unpublished data). Because

size-at-age is highly variable, it is beneficial for studies to be localized to control for spatial variation in diet, habitat and environment. Owing to the multi-faceted nature and expense of the IPHC surveys, a more time and cost efficient sampling strategy would facilitate deeper investigations of the mechanisms driving decreased size-at-age.

In May 2012, we initiated a port-sampling program in cooperation with the ADFG, Division of Sport Fish, Groundfish Research Program in Homer. Using contacts established through ADFG and protocols established by IPHC, we were able to expand upon their size-at-age sampling protocol to include diet (stomach contents and stable isotopes, $\delta^{13}C$ $\delta^{15}N$, in flesh) and disease (prevalence and intensity of *Ichthyophonus hoferi)*. This analysis focuses on the comparison of our size-at-age data to the data collected during IPHC stock assessment cruises.

The aim of this research was to compare the mean sizes-at-age of our sample, taken from the sport fishery in the port of Homer, to the mean sizes-at-age of the IPHC's survey sample. Port sample data were compared to the IPHC survey data for all of regulatory area 3A and for statistical area 261, the area encompassing lower Cook Inlet. In particular, we examined the age at which halibut attained the commercial exploitable size limit (81 cm). In most cases, the mean size-at-age of port sampled fish was significantly larger than both regional and local samples taken by the IPHC survey and port sampled halibut tended to reach exploitable size about two years sooner.

1.2 METHODS

Field Sampling

Halibut were sampled in the port of Homer in concert with the Alaska Department of Fish and Game port-sampling program. Located in lower Cook Inlet, Homer supports the largest Pacific halibut sport fishery in the United States (Meyer and Powers 2013; Figure 1). Many of the local fishers and charter fishing operations fillet their catch at fish processing stations and businesses located at the port (Figure 1.1). At these stations ADFG staff interview fishers and sample the catch. Once filleted, the carcasses are discarded in dumpsters, ultimately ground and dumped at sea. We collected *pre-dumpster, post-mortem* fork length and mouth gape measurements, determined sex and stomach contents, removed blind-side sagittal otolith for aging, and collected flesh tissue samples for diet and disease analyses from each filleted halibut carcass. Our aim was to sample at least 30 male and 30 female halibut in as many 10-centimeter fork length size bins as possible (< 39 cm, 40 – 49 cm, 50 – 59 cm, etc.) Fork length was measured for each fish to the nearest cm, and a visual evaluation of gonads was used to determine the sex following the 2012 Field Procedure Manual for the Southcentral Alaska Halibut and Groundfish Harvest Assessment Program of ADFG. When present, catch location (ADFG Statistical Area) was requested of the charter captain or private fisher.

Otolith Aging

Following IPHC aging protocols, the blind-side otolith of each fish was collected and cleared for at least three weeks in a glycerin solution of 5.5g thymol, 20ml 99.5% ethanol, 0.5 gallon glycerin and 0.5 gallon water (IPHC 2001). Once clear, otoliths were broken radially through the nucleus. The dorsal half was baked at 500^{o}F for 20 minutes in a conventional toaster oven. Otoliths were aged by counting dark concentric rings (winter growth) on the broken edge under 5X – 50X magnification using reflected light (Leica M60 stereomicroscope). If the broken

Figure 1.1. Port sampling in Homer, Alaska. Clockwise from top left: ADFG port sampler, fillet tables, carcasses in the dumpster and APU port samplers

surface was difficult to age, the unbaked proximal half was surface aged for agreement (Figure 1.2). Otolith agers were trained by IPHC age readers. Quality control standards set by the IPHC call for a minimum age agreement of 33% and minimum age agreement within one year of 75% for break and burn aging. To determine aging precision, every 10th otolith was sent to the IPHC for aging.

To assess precision of our otolith ages relative to those by IPHC staff, we calculated the percent of identical reads and those within 1 year, then compared them with a paired samples t-test. Further, residuals, r_i, were calculated by subtracting our read from the IPHC read ($r_i = IPHC_i - APU_i$). The mean absolute error (MAE) and the mean squared error (MSE) were both calculated to incorporate the bias and spread of the error distribution.

$$MAE = \frac{1}{n} \sum_{i=1}^{n} |r_i|$$

$$MSE = \frac{1}{n} \sum_{i=1}^{n} (r_i)^2$$

Where n is the number of otoliths.

Data Analysis

To determine if the mean size-at-age of the port sample from Lower Cook Inlet differed from regional Gulf of Alaska halibut, I compared them to samples collected during the IPHC's 2012 and 2013 southcentral Alaska stock assessment surveys (regulatory area 3A) and to only samples taken from Lower Cook Inlet (IPHC statistical area 261). Size-at-age comparisons were made by sex due to the strong sexual dimorphism in size (IPHC 1998). T-tests and Mann-Whitney U-tests were used to assess differences in mean sizes-at-age and only age groups with at least 10 fish samples were compared.

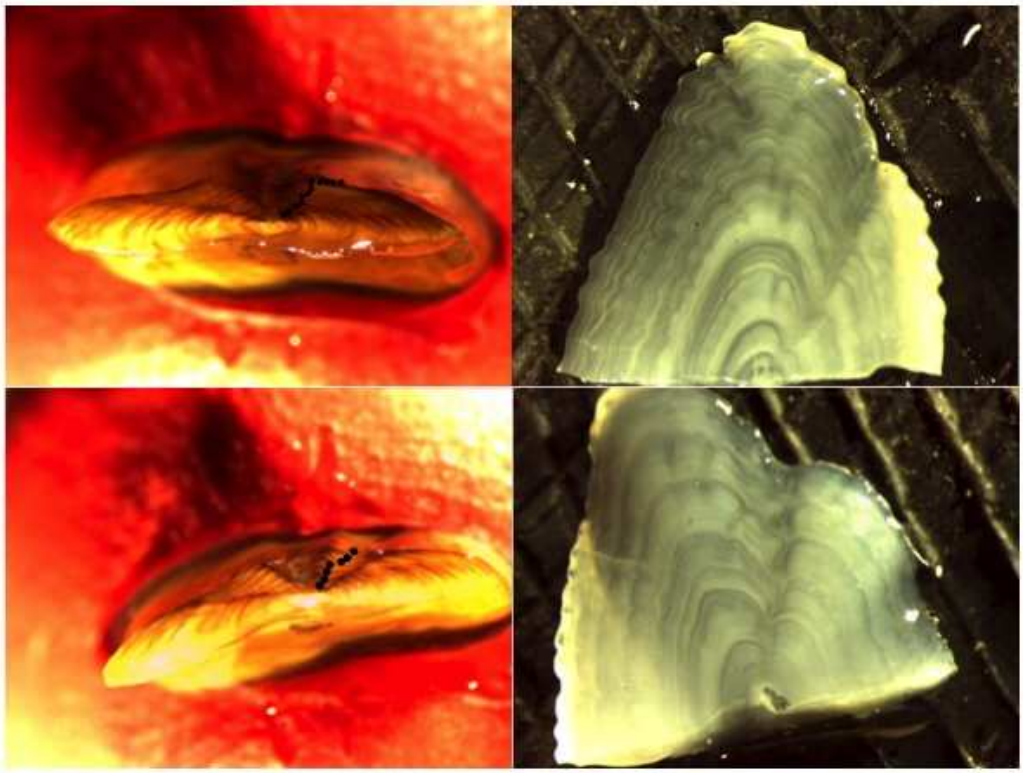

Figure 1.2. Otolith images. Top left is a cross section from a 12-year-old fish with annuli marked; the anterior half of this otolith is to the right. Bottom left is a cross section from a 7-year-old fish; the anterior haft of this otolith is also to the right.

1.3 RESULTS

Summary of APU sampling:

We sampled 429 fish in 2012 and 316 in 2013 for a total of 745. Of these, 481 females and 221 males were analyzed for size-at-age. Fish that were not analyzed had gonads removed, otoliths removed, or tails removed prior to collection, so size-at-age analyses were not possible.

Females ranged from 24 to 200 cm fork length and 2 to 25 years (Figures 1.3, 1.4). Target sample size (n = 30) was achieved for females in the 60, 70, 80, 90, 100 and 110 size bins. Male fish ranged from 41 to 107 cm fork length and 4 to 29 years (Figures 1.3, 1.4). Target sample size for males was reached in the 60, 70 and 80 cm size bins.

Otolith agreement with IPHC

The head age reader at IPHC did a second read on 79 otoliths for age verification (11.3% of otoliths). Age agreement was 55.7%. Age agreement ± 1 year was 83.5% with 10 otoliths aged by APU one year older and 12 otoliths aged by APU one year younger (Figure 1.5). There was no significant difference between our ages (mean = 11.8, SD = 4.50) and IPHC ages (mean = 11.9, SD = 4.58) ($n = 79$, $t = 0.60$, $p > 0.05$, two-way paired samples t-test). The mean absolute error was 0.747 and the mean squared error was 1.709.

Females: IPHC area 3A

During 2012 and 2013, I sampled 481 female halibut. These data were compared to 1,891 females sampled by the IPHC in area 3A during the same years (Figure 1.6). For all ages where sample size was greater than 10 (ages 6 – 14), mean size-at-age in the port sample was

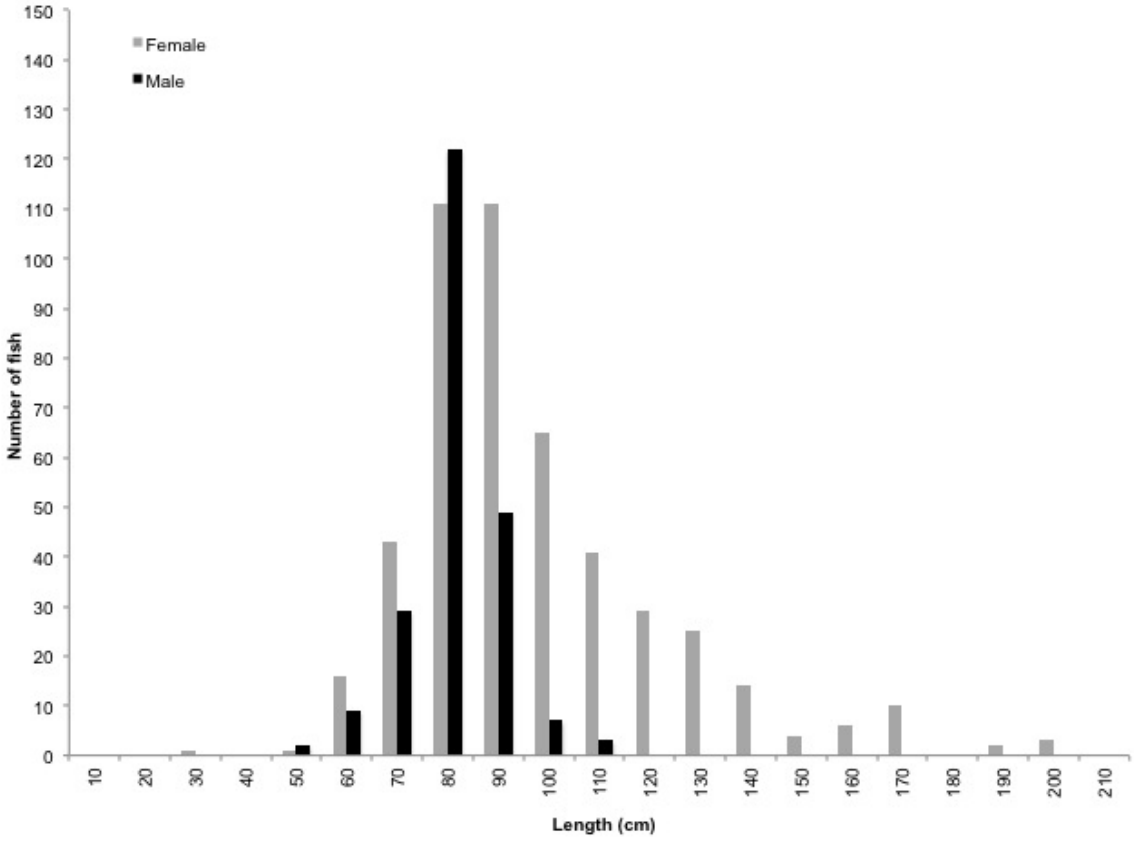

Figure 1.3. Length distribution of halibut from port sampling (2012 and 2013 combined). Females ranged from 24 – 200 cm. Males ranged from 41 – 107 cm.

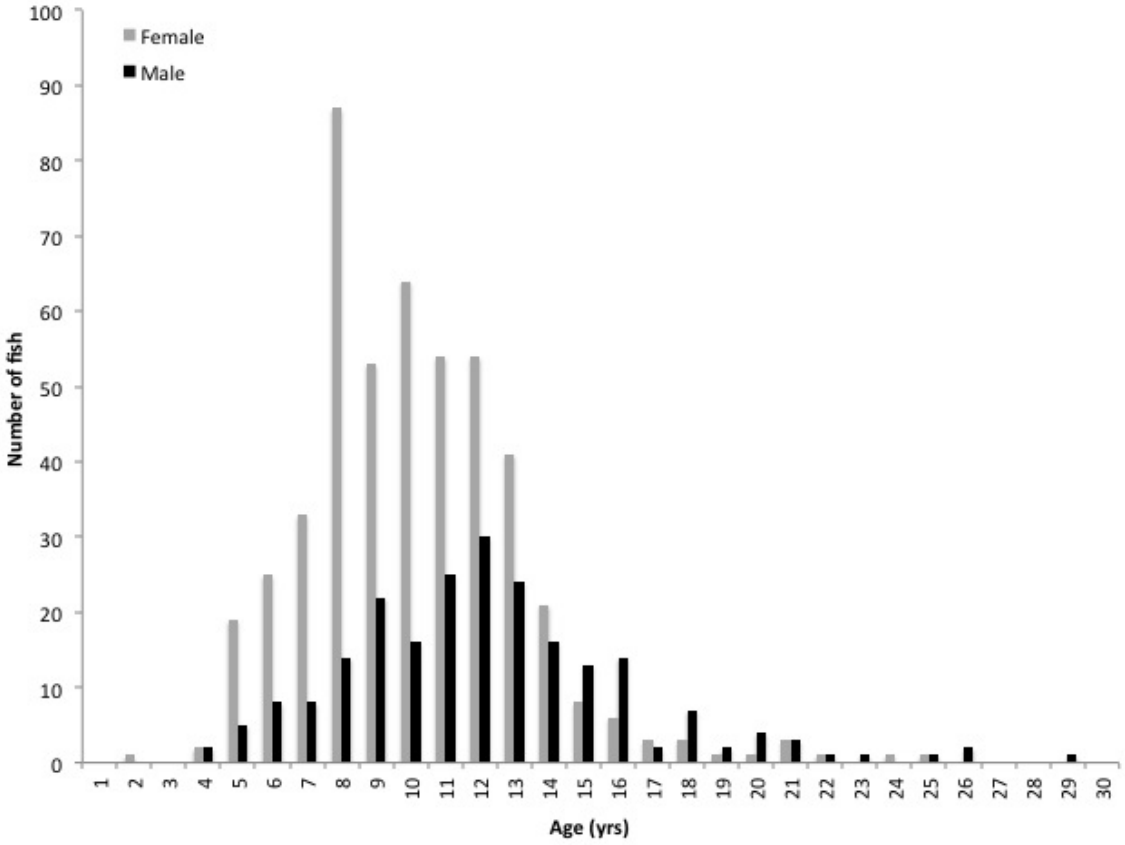

Figure 1.4. Age distribution of males and females from port sampling (2012 and 2013 combined). Female ages ranged from 2 – 25 years. Males ranged from 4 – 29 years.

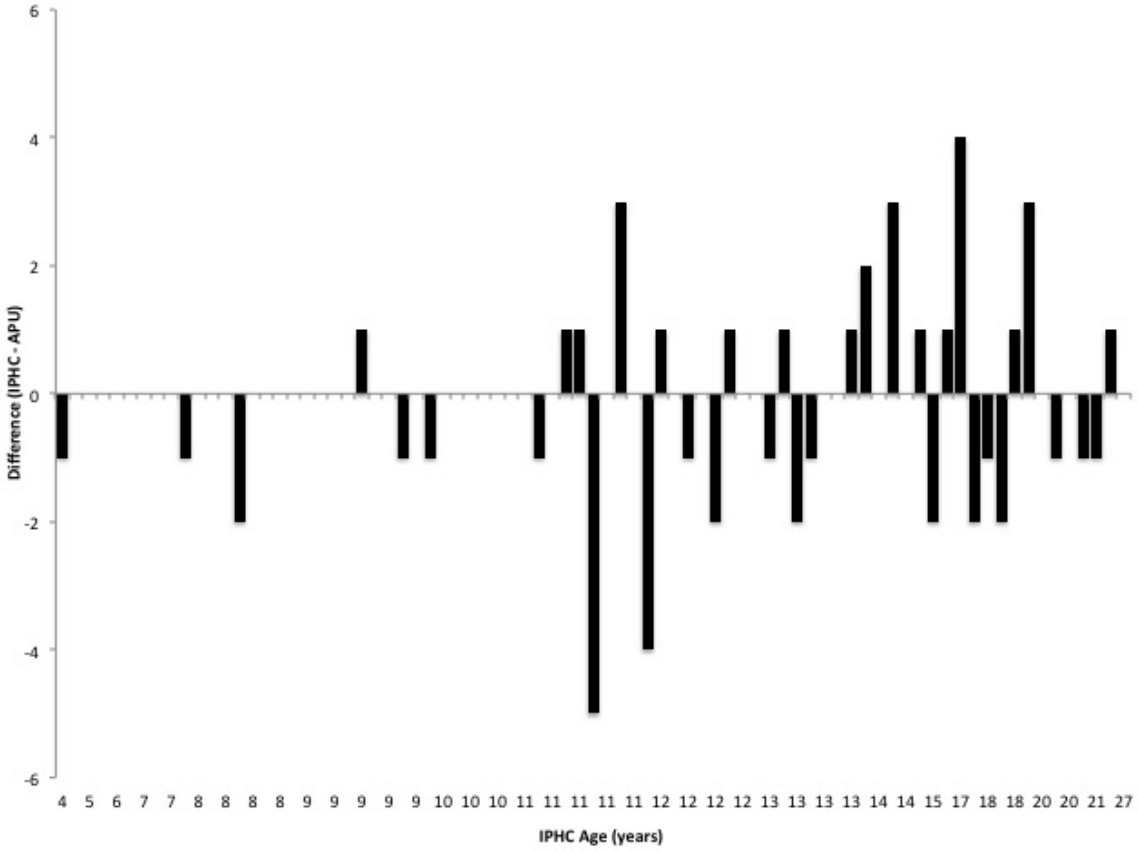

Figure 1.5. APU and IPHC ages for the same otolith. Bars above y = 0 indicate otoliths that were aged older by IPHC than APU; points below were aged younger by IPHC. Agreement was 55.7% (83.5% ± 1 year).

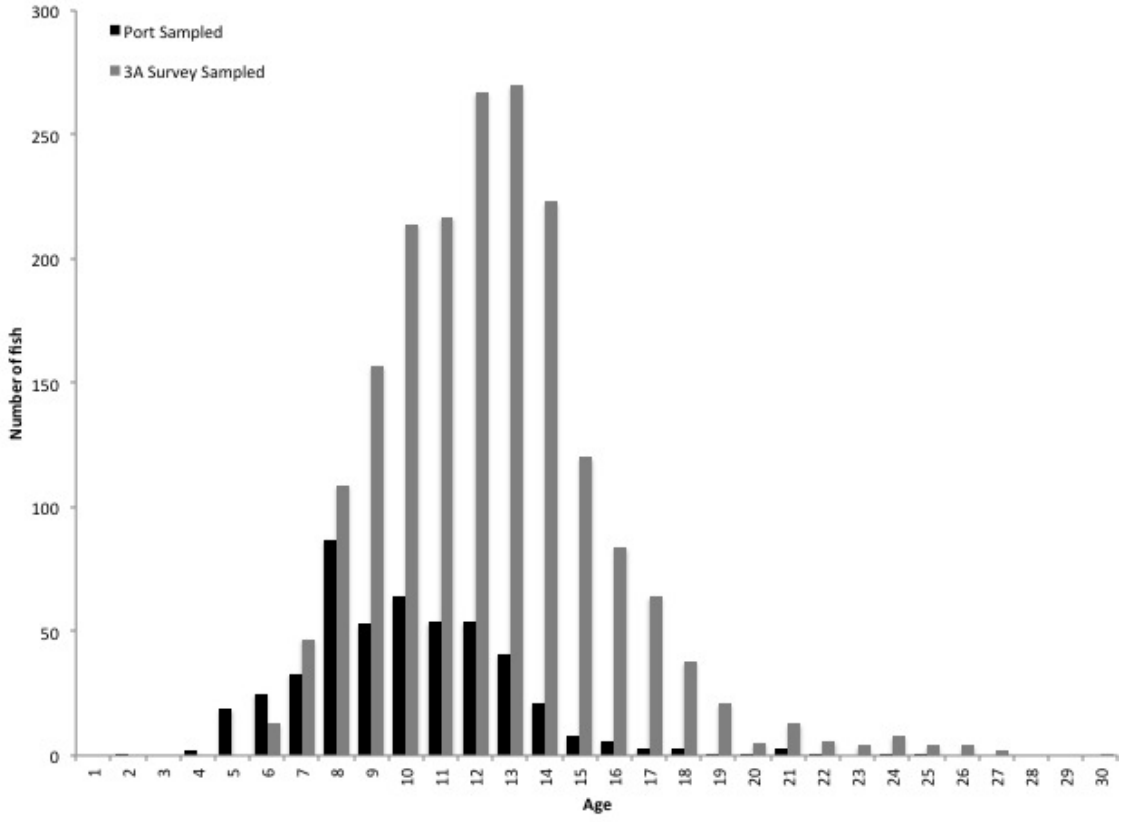

Figure 1.6. Distribution of ages of port sampled and 3A survey sampled female halibut (2012 and 2013 combined) showing a younger population structure in the port sample.

significantly larger than in the survey sample (two-way t-tests for independent samples, $p < 0.05$; Mann-Whitney Rank U-tests, $p < 0.01$; Figure 1.7, 1.8; Table 1.1). 50% of females attained 81cm by 8 years in the port sample compared to 11 years in the survey sample. 100% of sampled fish were 81cm by age 15 in the port sample and 19 in the survey sample (Figure 1.9).

Males: IPHC area 3A

During 2012 and 2013, I sampled 221 male halibut. These data were compared to 1,371 males sampled by the IPHC in area 3A during the same years (Figure 1.10). Nine ages had sample sizes greater than 10 (ages 8 – 16); mean size-at-age in the port sample was larger than the survey sample for 7 of those ages. This was significant for ages 8, 9, 10 and 11 (two-way t-tests for independent samples, $p < 0.05$; Mann-Whitney Rank U-tests, $p < 0.05$; Figure 1.11, 1.12; Table 1.2). The age at which 50% of males attained 81 cm was 15 years in the port sample compared to 17 years in the survey sample. 100% of sampled fish were 81cm by age 19 in the port sample and 23 in the survey sample, with the exception of one fish aged 26 years (Figure 1.13).

Females: IPHC area 261

Port sample data were also compared to the subset of IPHC data from Lower Cook Inlet (n = 144; figure 1.14). Six age classes (9 – 14) had at least 10 samples from both groups. Mean size-at-age in the port sample was larger for all years; t-tests had significant results for ages 9 – 13 ($p < 0.01$) while u-tests had significant results for ages 9 – 12 ($p < 0.05$; Figure 1.15, 1.16; Table 1.3). The age at which 50% of females reached 81 cm was 10 in the survey sample; 100% of fish were 81cm by 14 years (Figure 1.9).

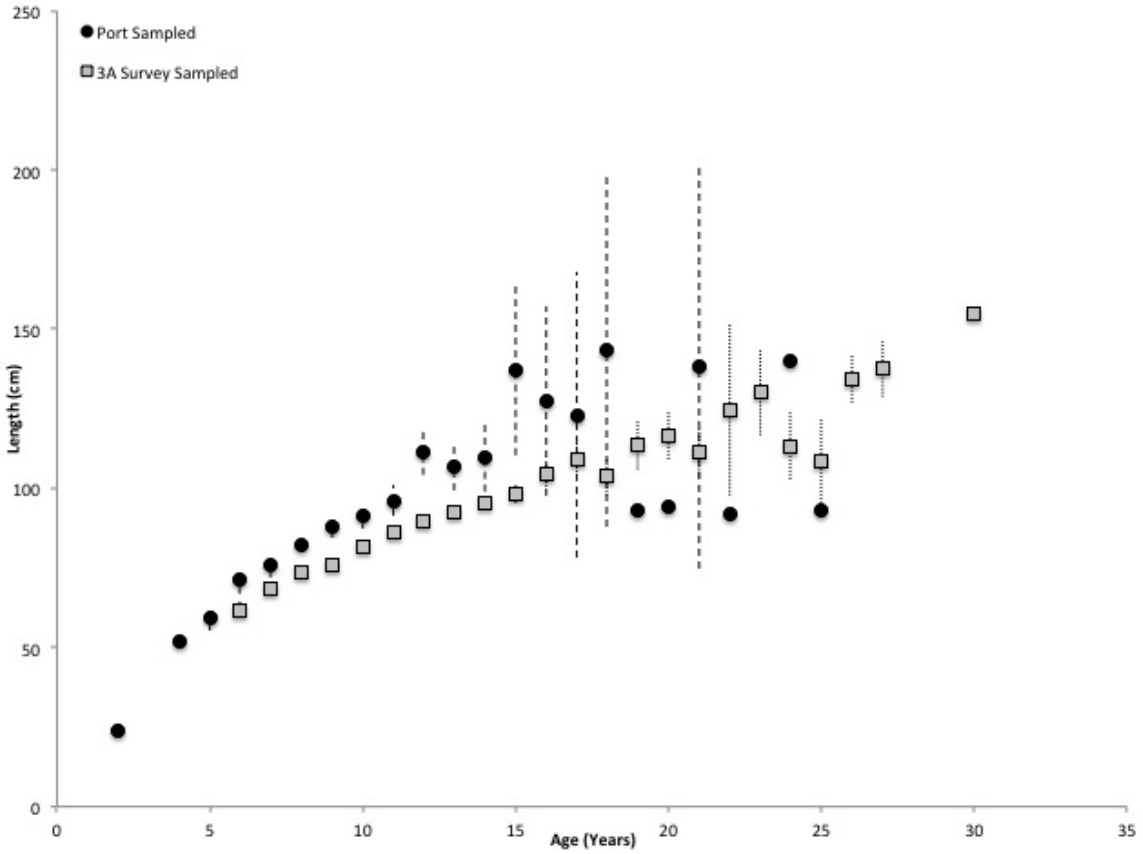

Figure 1.7. Mean length-at-age of females from port sampled and 3A survey sampled halibut with 95% confidence intervals showing greater mean length-at-age for most ages in the port sampled fish.

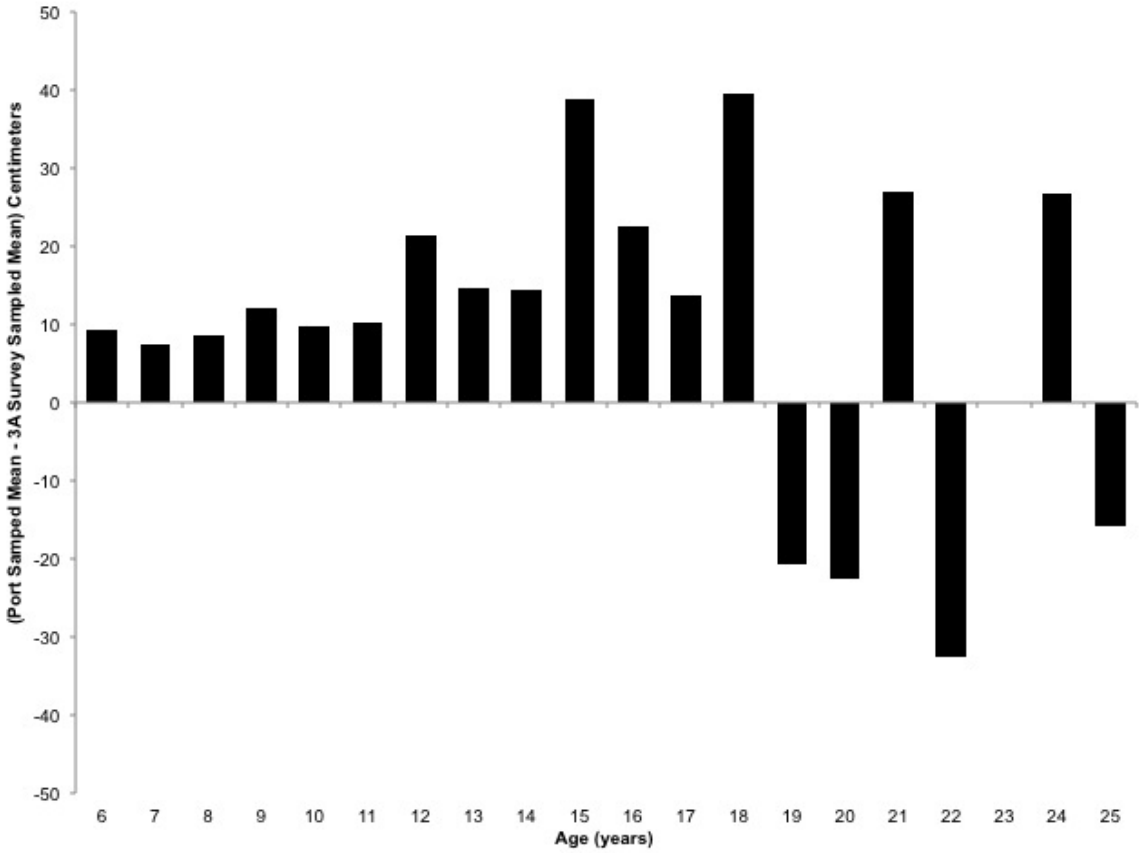

Figure 1.8. Difference between mean length-at-age of females taken by port sampling and 3A survey sampling. Difference is calculated by subtracting the survey sampled mean from the port sampled mean. For all samples where port sampled n = 5, port sampled mean length-at-age was greater.

Table 1.1. Mean length-at-age analysis for female halibut sampled by APU (port sampling) and IPHC Area 3A (survey sampling). Two tailed t-test for independence with unequal variance (T) and Mann-Whitney rank correlation test (U) results and associated p-values are reported. All ages with significant results are grey.

Age	APU 3A Mean	APU 3A N	IPHC 3A Mean	IPHC 3A N	T	p	U	p
2	24.0	1						
3								
4	52.0	2						
5	59.3	19						
6	71.2	25	61.8	13	3.6816	0.0008	265.0	0.0011
7	75.6	33	68.3	47	3.4290	0.0012	1106.0	0.0011
8	82.3	87	73.6	109	5.4820	0.0000	6866.5	0.0000
9	88.0	53	75.9	157	5.9157	0.0000	6558.5	0.0000
10	91.2	64	81.5	214	4.3409	0.0000	9288.5	0.0000
11	96.2	54	86.0	217	3.9150	0.0002	7762.0	0.0002
12	111.2	54	89.8	267	5.7832	0.0000	10775.5	0.0000
13	107.0	41	92.4	270	3.6410	0.0007	7506.0	0.0002
14	109.6	21	95.3	223	2.5983	0.0168	3172.5	0.0066
15	137.0	8	98.1	120	2.8455	0.0249	818.0	0.0004
16	127.2	6	104.7	84	1.4663	0.2025	342.0	0.1515
17	123.0	3	109.2	64	0.5985	0.6103	113.5	0.6146
18	143.3	3	103.7	38	1.3872	0.2997	87.5	0.1332
19	93.0	1	113.7	21				
20	94.0	1	116.6	5				
21	138.3	3	111.5	13	0.8220	0.4975	25.0	0.5214
22	92.0	1	124.7	6				
23			130.3	4				
24	140.0	1	113.3	8				
25	93.0	1	108.8	4				
26			134.3	4				
27			137.5	2				

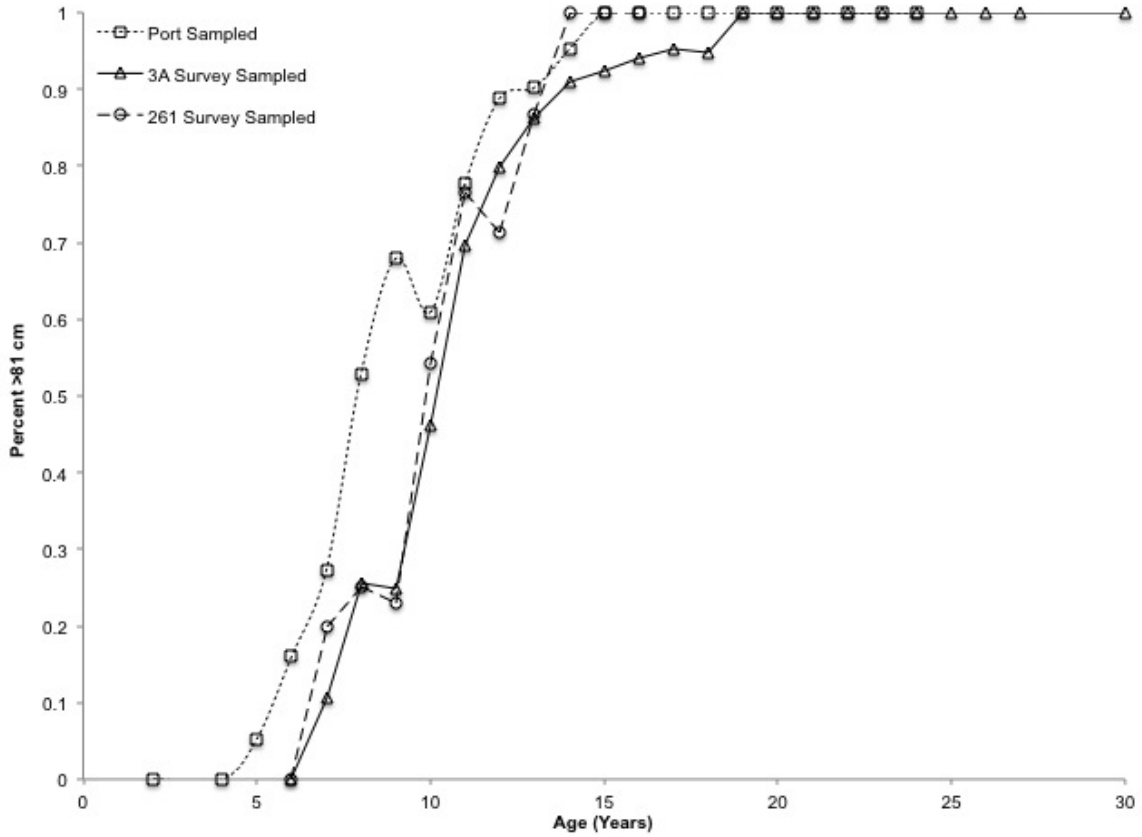

Figure 1.9. Percent of female fish by age that have reached 81 cm. The port sampled halibut had a higher proportion of fish that were at least 81 cm than the 3A survey sampled halibut for every age and for all but one age (Age 14) of the 261 survey sampled halibut.

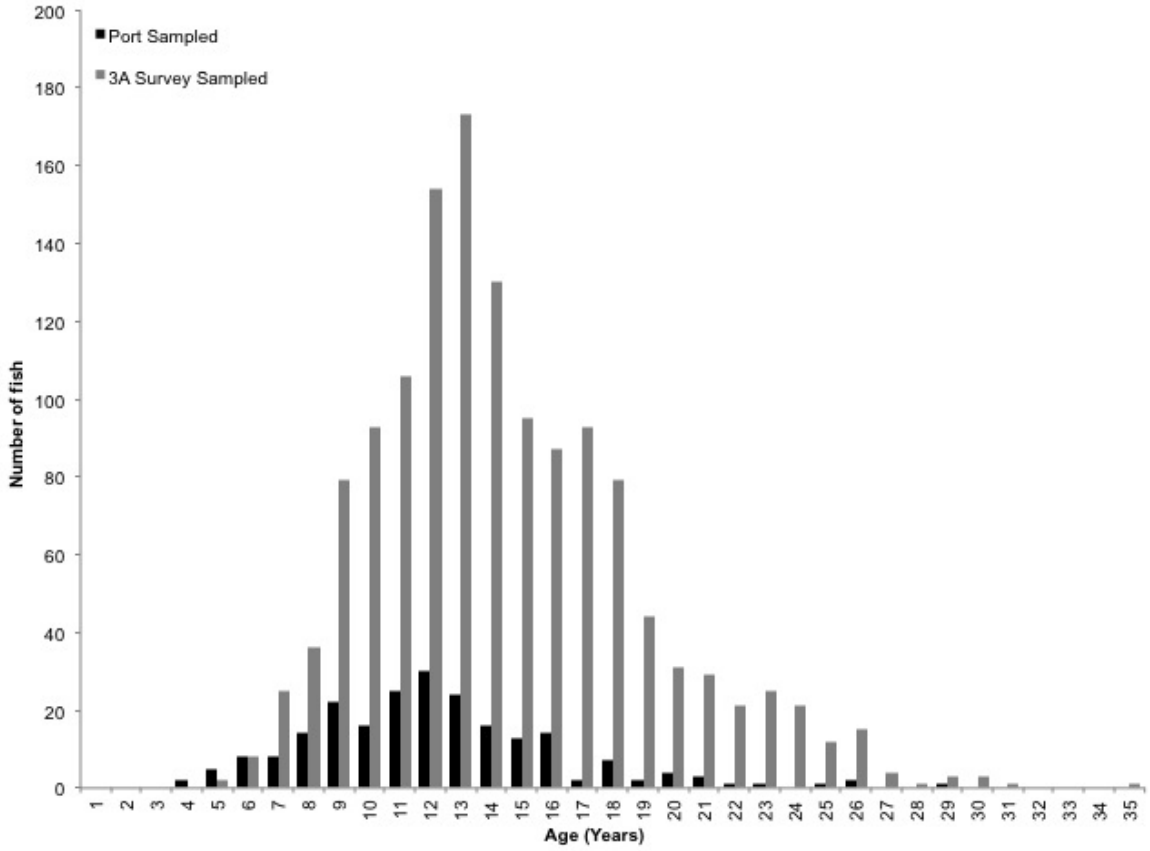

Figure 1.10. Distribution of male halibut ages from port sampled and 3A survey sampled halibut (2012 and 2013 combined) showing a younger population structure in the port sample.

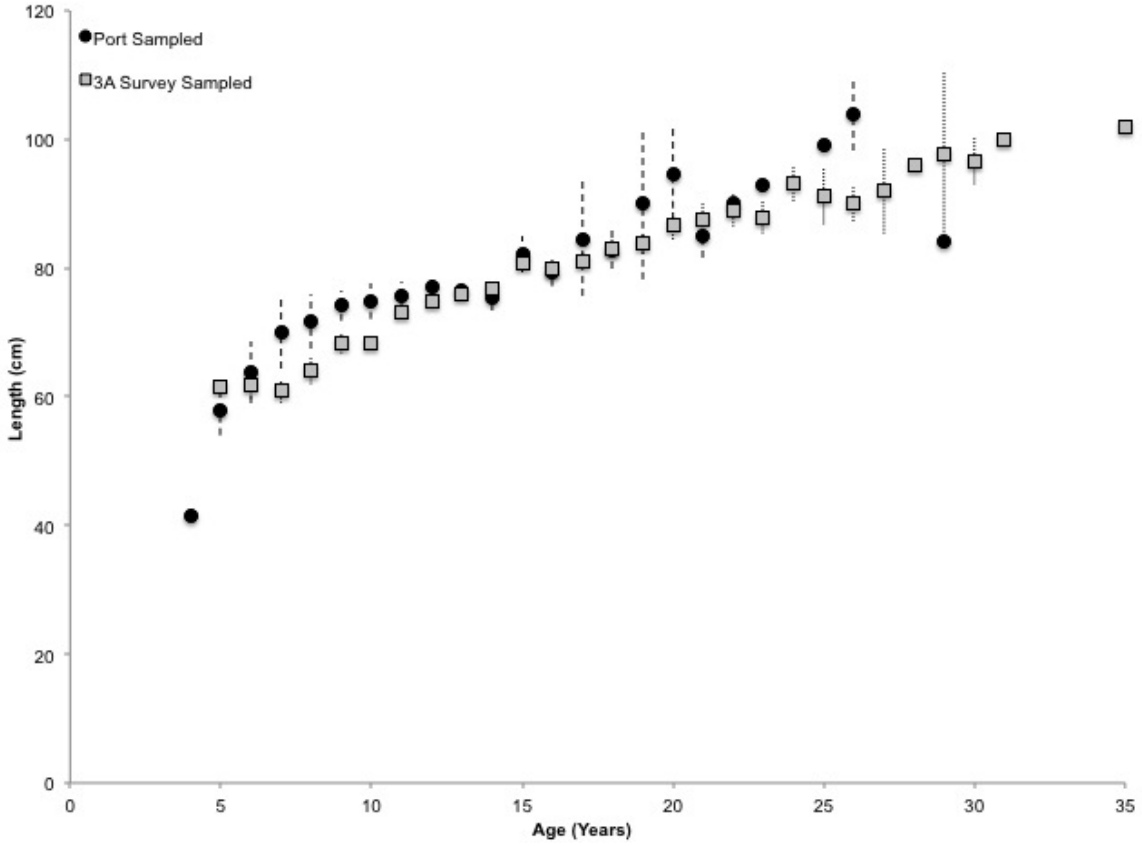

Figure 1.11. Mean length-at-age of males from port sampled and 3A survey sampled fish with 95% confidence intervals. Mean length-at-age in the port sample was larger for younger fish and similar for most other age groups.

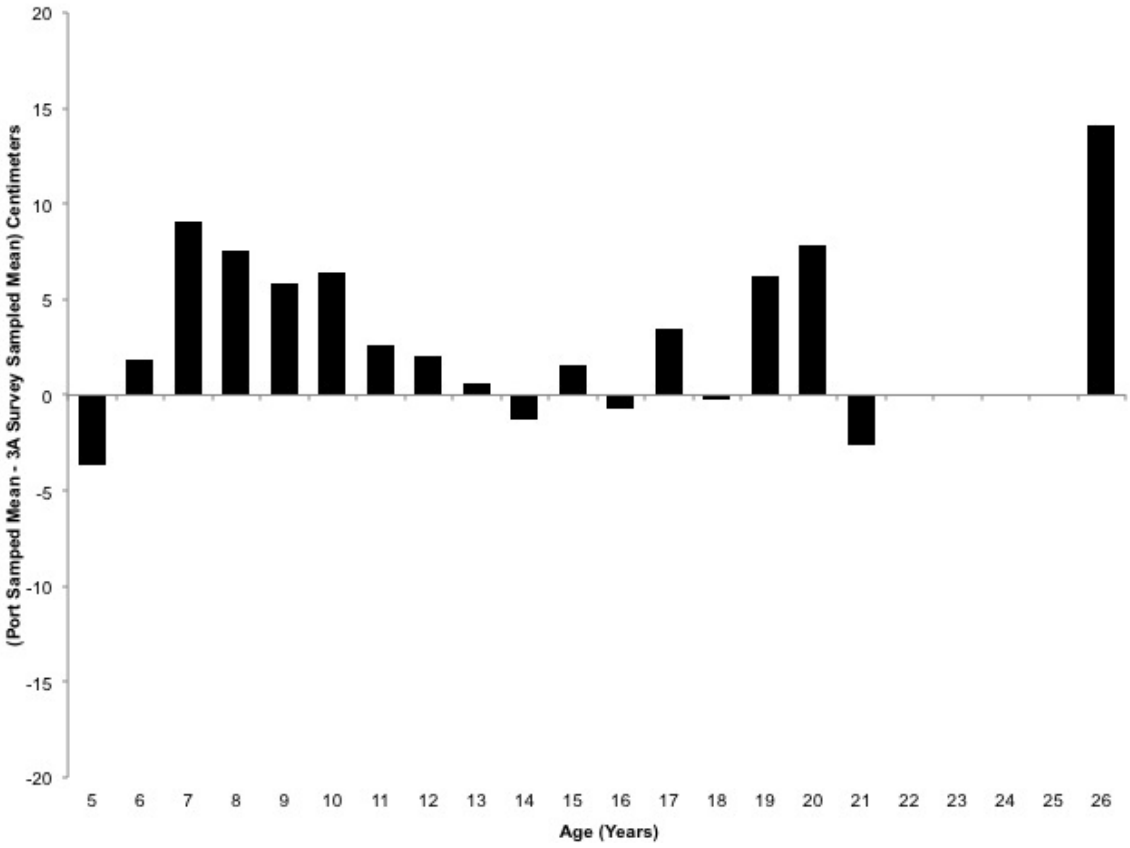

Figure 1.12. Difference between mean length-at-age of females taken by port sampling and 3A survey sampling. Difference is calculated by subtracting the survey sampled mean from the port sampled mean.

Table 1.2. Mean length-at-age analysis for male halibut sampled by APU (port sampling) and IPHC Area 3A (survey sampling). Two tailed t-test for independence with unequal variance (T) and Mann-Whitney rank correlation test (U) results and associated p-values are reported. All ages with significant results are grey.

Age	APU 3A Mean	APU 3A N	IPHC 3A Mean	IPHC 3A N	t	p	U	p
4	41.5	2						
5	57.8	5	61.5	2	1.8523	0.1376	8.0	0.3810
6	63.8	8	61.9	8	0.6995	0.5002	36.0	0.7209
7	70.0	8	60.9	25	2.9662	0.0158	168.5	0.0026
8	71.7	14	64.2	36	3.0692	0.0061	350.5	0.0071
9	74.1	22	68.3	79	4.1442	0.0002	1337.0	0.0001
10	74.8	16	68.4	93	4.2396	0.0004	1185.0	0.0001
11	75.7	25	73.1	106	2.0554	0.0470	1728.5	0.0175
12	77.0	30	74.9	154	1.8100	0.0770	2912.5	0.0235
13	76.4	24	75.8	173	0.6727	0.5041	2465.0	0.1379
14	75.4	16	76.7	130	1.0651	0.2970	1099.0	0.7172
15	82.2	13	80.7	95	0.9846	0.3379	739.0	0.2571
16	79.3	14	80.0	87	0.5474	0.5888	640.0	0.7678
17	84.5	2	81.0	93	0.7604	0.5861	121.0	0.5026
18	82.9	7	83.1	79	0.1490	0.8845	298.0	0.7476
19	90.0	2	83.8	44	1.0230	0.4928	68.0	0.2338
20	94.5	4	86.6	31	2.0400	0.1110	106.0	0.0197
21	85.0	3	87.7	29	1.2676	0.2737	55.0	0.4968
22	90.0	1	88.9	21				
23	93.0	1	87.9	25				
24			93.1	21				
25	99.0	1	91.3	12				
26	104.0	2	89.9	15	4.2533	0.1470	29.0	0.0294
27			92.0	4				
28			96.0	1				
29	84.0	1	97.7	3				
30			96.7	3				
31			100.0	1				
32								
33								
34								
35			102.0	1				

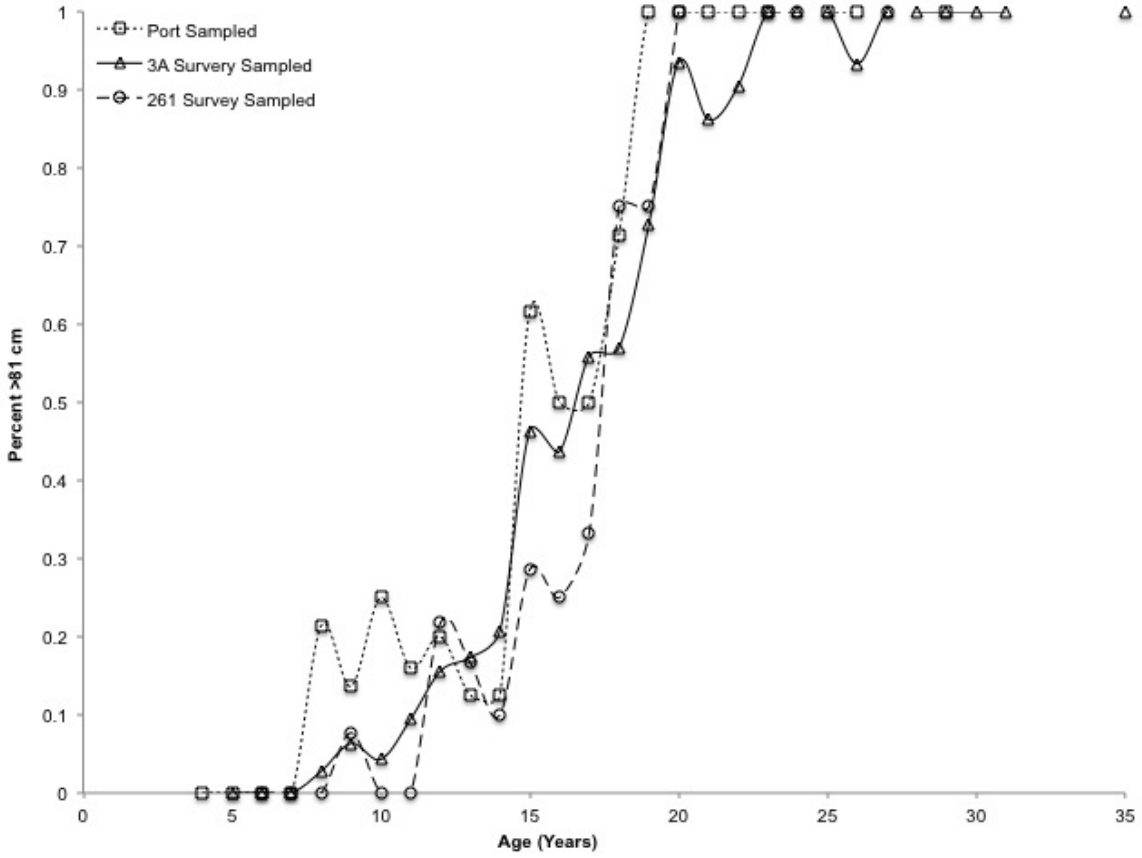

Figure 1.13. Percent of male fish by age that have reached 81 cm. The port sample had a higher proportion of fish that were at least 81 cm 3A survey sample for most younger ages.

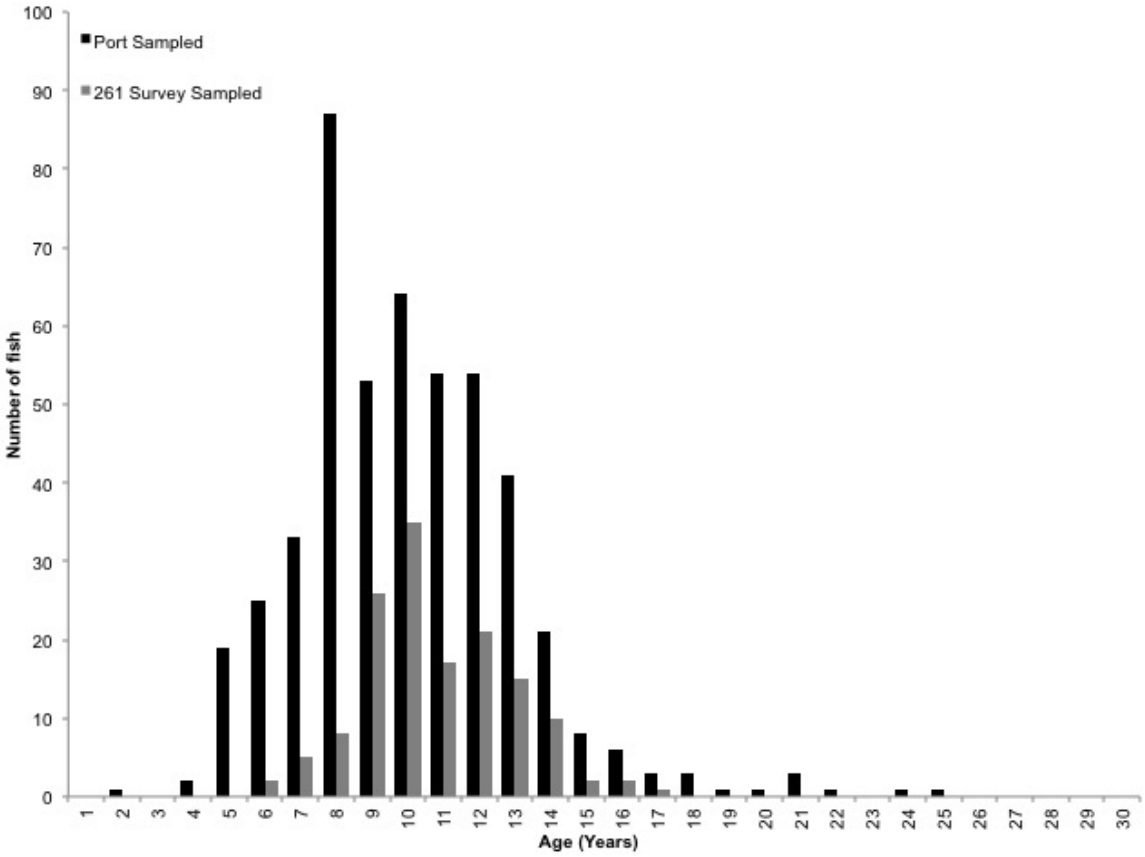

Figure 1.14. Distribution of ages of port sampled and 261 survey sampled female halibut (2012 and 2013 combined).

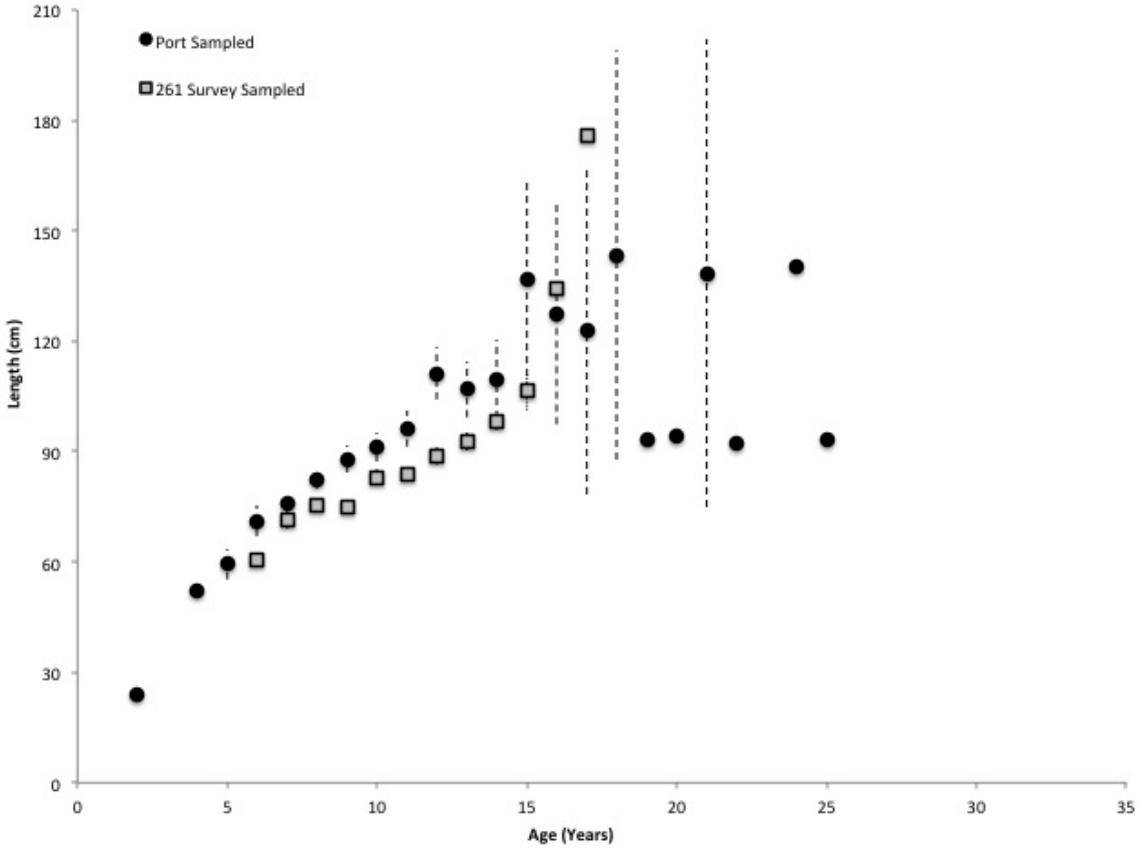

Figure 1.15. Mean length-at-age of females from port sampled and 3A survey sampled halibut with 95% confidence intervals showing greater mean length-at-age for most ages in the port sampled fish.

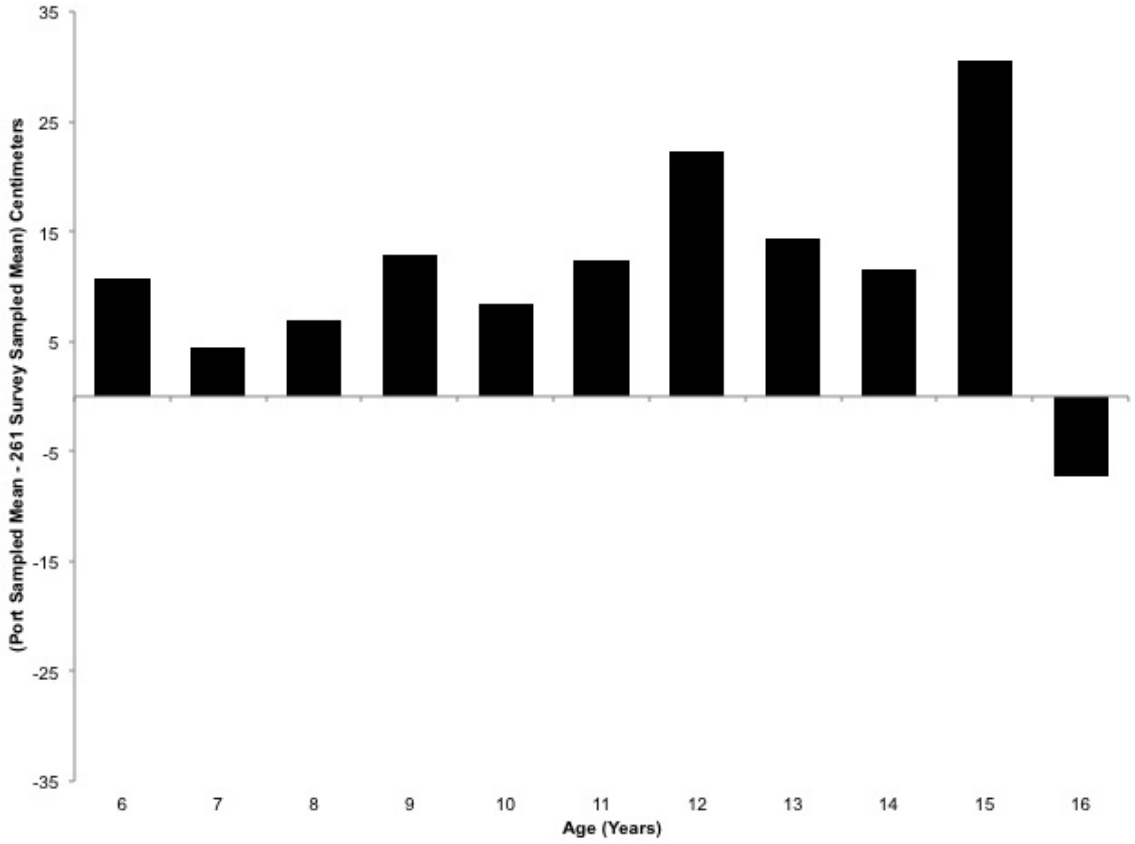

Figure 1.16. Difference between mean length-at-age of females from port sampling and survey sampling in statistical area 261. Difference is calculated by subtracting the survey sampled mean from the port sampled mean. Port sampled mean length-at-age was consistently larger than survey sampled mean length-at-age.

Table 1.3. Mean length-at-age analysis for female halibut sampled by APU (port sampling) and IPHC statistical Area 261 (survey sampling). Two tailed t-test for independence with unequal variance (T) and Mann-Whitney rank correlation test (U) results and associated p-values are reported. All ages with significant results are grey.

Age	APU Mean	APU N	IPHC Mean	IPHC N	T	p	u	p
2	24.0	1						
3								
4	52.0	2						
5	59.3	19						
6	71.2	25	60.5	2	4.7024	0.0001	47.0	0.0342
7	75.6	33	71.2	5	0.9278	0.3893	102.5	0.3992
8	82.3	87	75.4	8	2.1260	0.0594	484.5	0.0668
9	88.0	53	75.1	26	5.3240	0.0000	1109.5	0.0000
10	91.2	64	82.9	35	3.0281	0.0032	1431.5	0.0222
11	96.2	54	83.8	17	4.2725	0.0001	641.5	0.0129
12	111.2	54	88.9	21	5.0198	0.0000	866.0	0.0003
13	107.0	41	92.7	15	2.8014	0.0073	409.5	0.0589
14	109.6	21	98.0	10	1.7881	0.0842	124.5	0.4172
15	137.0	8	106.5	2	1.2828	0.3281	13.0	0.2667
16	127.2	6	134.5	2	0.4538	0.6659	8.0	0.6429
17	123.0	3	176.0	1				
18	143.3	3						
19	93.0	1						
20	94.0	1						
21	138.3	3						
22	92.0	1						
23								
24	140.0	1						
25	93.0	1						

Males: IPHC area 261

For males in area 261 (IPHC n = 127), age classes 9, 11 – 14 had at least 10 fish in samples (Figure 1.17). Fish sampled by port sampling were larger for ages 9, 11, 12 and 13. Only ages 9 and 11 were significant (t-test and U-test, $p < 0.01$; Figures 1.18, 1.19; Table 1.4). The age at which at least 50% of male fish reached 81 cm was 18 in the IPHC 261 sample; 100% of fish were 81cm by 20 years (Figure 1.13).

1.4 DISCUSSION

Mean sizes-at-age in this study were significantly different from the IPHC survey data for all female fish age classes with sample sizes larger than 10. Additionally, the mean sizes of female port sampled fish were larger for every age with a sample size of at least 2. For all ages of females, the proportion of port samples that had attained the 81 cm mark was greater than the proportion of survey samples. Male fish ages 6 – 14 were larger on average in the port sample; for age classes older than 14 there were an approximately equal number of port and survey age classes with larger means. Male fish in the port sample also tended to reach the 81 cm mark sooner than the survey fish; however, these data were much more variable than the female data.

While these results indicate that there are population level differences between the fish we sampled and the survey sampled fish in 3A and 261, our size-stratified sampling strategy may impact the results. Though age-at-length is not as commonly discussed in fisheries research, in this case, it may be a better analysis tool. However, initial analyses show that the fish in the port sample were growing significantly faster than the survey sampled fish in 3A (Table 1.5). Further, use of the sport fishery to sample fish likely resulted in an inflated size-at-age relative to

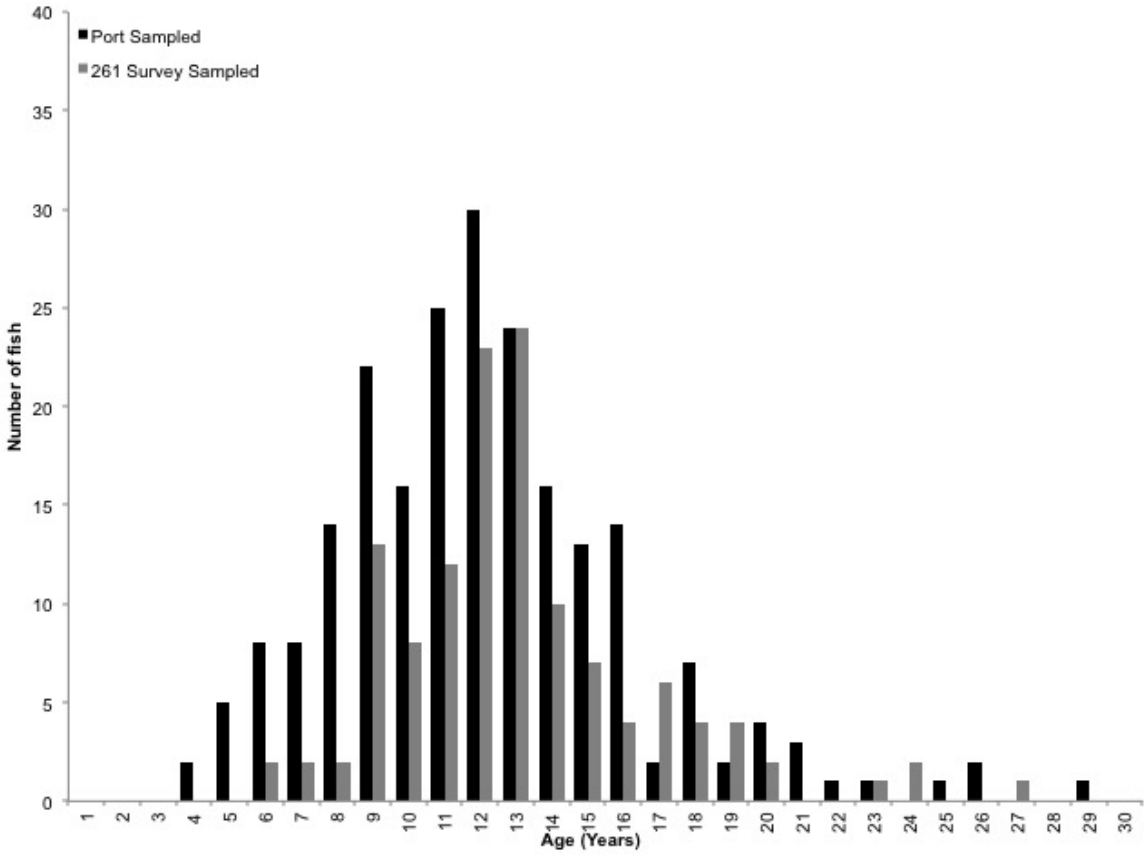

Figure 1.17. Age distribution of male halibut that were port sampled and survey sampled in area 261 (2012 and 2013 combined) showing a greater proportion of young fish in the APU sample.

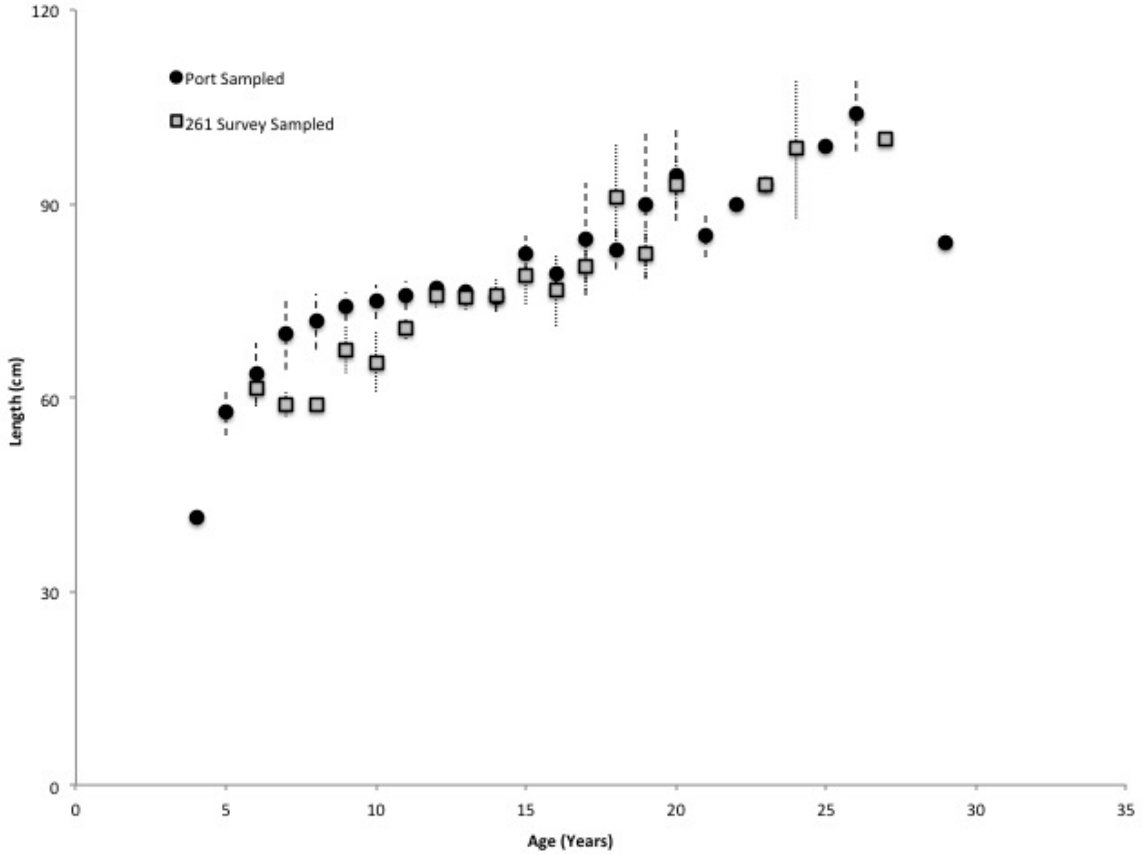

Figure 1.18. Mean length-at-age of males from port sampling and survey sampling in area 261 with 95% confidence intervals.

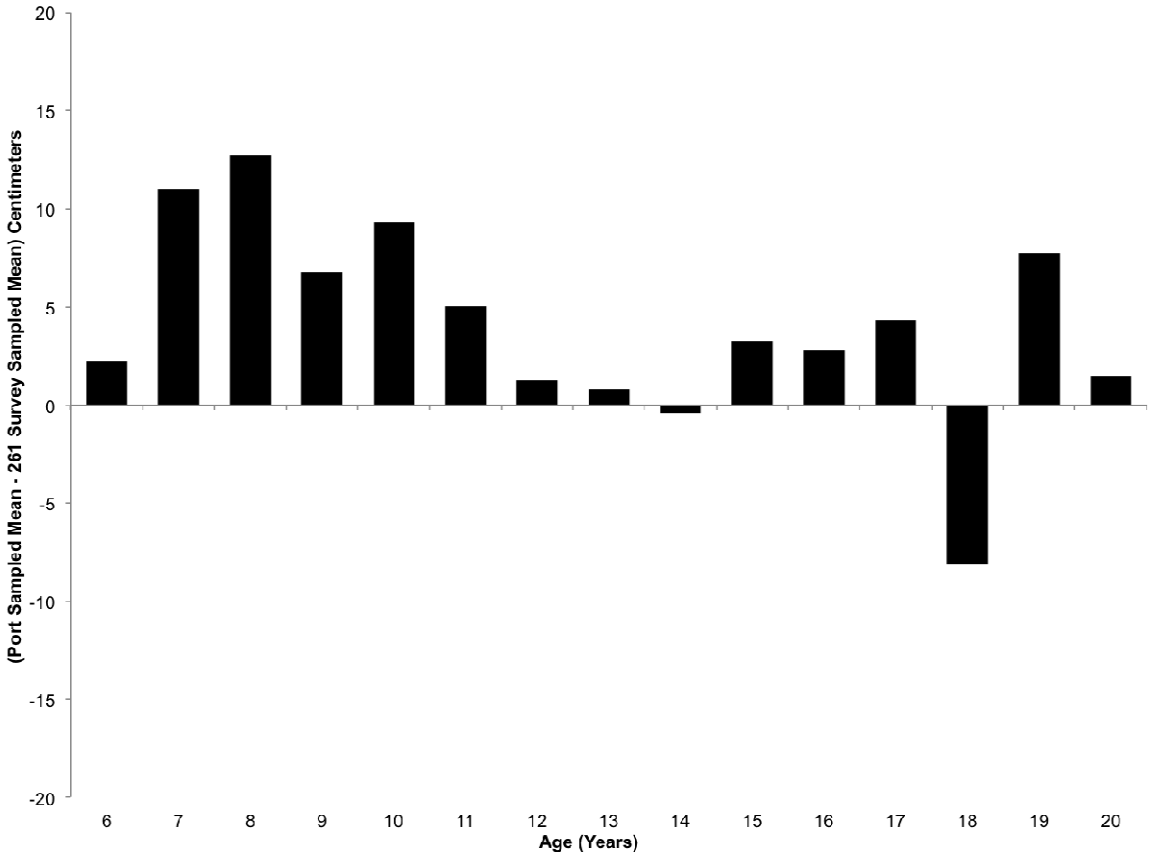

Figure 1.19. Difference between mean length-at-age of males from port sampling and survey sampling in statistical area 261. Difference is calculated by subtracting the survey sampled mean from the port sampled mean. Port sampled mean length-at-age was consistently larger than survey sampled mean length-at-age

Table 1.4. Mean length-at-age analysis for male halibut sampled by APU and IPHC Statistical Area 261. Two tailed t-test for independence with unequal variance (t) and Mann-Whitney rank correlation test (u) results and associated p-values. All ages with significant results are greyed.

Age	APUMean	APUN	IPHCMean	IPHCN	T	p	U	p
4	41.5	2						
5	57.8	5						
6	63.8	8	61.5	2	0.7855	0.4579	8.5	0.8889
7	70.0	8	59.0	2	3.5756	0.0072	14.0	0.1778
8	71.7	14	59.0	2	5.8124	0.0001	28.0	0.0167
9	74.1	22	67.4	13	3.0818	0.0055	235.0	0.0012
10	74.8	16	65.5	8	3.3829	0.0054	110.0	0.0036
11	75.7	25	70.7	12	3.5212	0.0013	238.5	0.0031
12	77.0	30	75.7	23	0.9187	0.3626	399.5	0.1999
13	76.4	24	75.6	24	0.6195	0.5390	333.0	0.3632
14	75.4	16	75.8	10	0.2549	0.8014	82.0	0.9382
15	82.2	13	79.0	7	1.1478	0.2778	62.5	0.1827
16	79.3	14	76.5	4	0.9169	0.4111	36.5	0.3817
17	84.5	2	80.2	6	0.8943	0.5355	9.5	0.2857
18	82.9	7	91.0	4	1.7691	0.1516	23.0	0.1091
19	90.0	2	82.3	4	1.2321	0.4340	7.0	0.2667
20	94.5	4	93.0	2	0.3594	0.7375	5.0	0.8000
21	85.0	3						
22	90.0	1						
23	93.0	1	93.0	1				
24			98.5	2				
25	99.0	1						
26	104.0	2						
27			100.0	1				
28								
29	84.0	1						

Table 1.5. Age-at-length t-test results for APU and IPHC Area 3A. Age difference = IPHC Age – APU age. In all cases, APU fish were younger for their size. This was significant for all but one length bin. In two of these length bins, the size was also significantly larger for APU sampled fish.

Females											
Length	APU Mean Age	APU N	APU SD	IPHC Mean Age	IPHC N	IPHC SD	Age Difference	t	p age	p size	Size Difference
50	5.4	17	1.18	7.1	16	0.85	1.7	4.590	0.0001	0.0000	3.7
60	6.6	35	1.65	8.5	137	1.43	1.9	6.866	0.0000	0.0352	1.1
70	8.9	103	1.68	10.6	369	2.05	1.7	7.617	0.0000	0.1674	
80	9.8	122	2.30	12.1	578	2.19	2.2	10.178	0.0000	0.0648	
90	11.5	63	3.60	13.4	366	2.50	1.9	5.077	0.0000	0.6705	
100	11.0	42	2.35	14.0	202	2.67	3.0	6.836	0.0000	0.3198	
110	10.9	28	1.82	15.1	96	3.23	4.2	6.518	0.0000	0.8774	
120	11.9	30	2.08	16.0	58	3.50	4.1	5.911	0.0000	0.5046	
130	14.0	12	2.80	16.2	37	4.29	2.2	1.692	0.0973		

Males											
60	8.7	28	2.45	10.4	243	2.37	1.7	3.546	0.0005	0.7332	
70	11.6	116	2.38	13.3	573	2.47	1.7	6.754	0.0000	0.0932	
80	14.4	55	4.00	17.0	364	3.75	2.6	4.669	0.0000	0.2260	

the true population. Sport fishers tend to target large halibut and it is possible that many of the smaller fish that are released in this process have more similar individual growth to the fish seen in IPHC surveys. This type of sampling is therefore limited in its ability to reflect true populations.

Observed differences may also be due to a combination of localized differences in temperature, depth, forage and migration (Helfman *et al.* 2009, Clark *et al*. 1999, Clark and Hare 2002, Orlov *et al.* 2011, IPHC 2013b). Most of the fish that were used in our study were from nearshore waters that are not included in the IPHC's annual survey. Because nearshore waters are shallower, water temperatures at depth are usually warmer than the water temperatures offshore (http://portal.aoos.org/gulf-of-alaska.php); however, these data are somewhat confounded by glacial runoff. Optimum water temperature allows for more efficient metabolism in fishes and thereby increases growth. In other fish species (e.g. Atlantic cod, salmon) variation in growth is correlated with changes in temperature (Brander 1995, Campana *et al.* 1995, Hinch *et al.* 1995, Cox and Hinch 1997). Further, these waters are likely to be more productive due to increased light penetration (Morrissey and Sumich 2012). Differences in the quantity or quality of forage may also be a result of differences in suitable habitat for forage fish, inter- and intraspecific competition, and seasonal migrations of forage fish. Research on the migratory patterns of halibut is still in early stages (IPHC 2006b, Webster *et al.* 2013). Size-at-age may depend on the duration and frequency of migration and where the fish spent their juvenile years. In order to fully understand the role that each of these factors plays in halibut size-at-age performance, more intensive localized sampling is necessary.

Recently, in addition to reduced harvest allocations for commercial fishers, sport fishers have also seen reductions in the Guideline Harvest Level (GHL) of the charter fleet. These changes are applied to broad management regions and have included limits on number of fish, size of fish (maximum sizes and slot limits), and ability of charter captains and crews to fish while guiding (Meyers and Powers 2013). The localized nature of size-at-age demonstrated here may mean that in some areas the reduction in GHL is not necessary.

Pacific halibut is one of the most economically important fishes in the state of Alaska (IPHC 2013b, NMFS 2013, McDowell Group 2013), and the exploitable biomass in southcentral Alaska is currently low due to decreasing size-at-age since the late 1970s. As Pacific halibut size-at-age remains low, it is imperative that a cost and time efficient sampling strategy is developed to assess potential mechanisms causing slow growth. Localized studies of size-at-age mechanisms are needed to establish baseline data and distinguish amongst factors driving size-at-age in different regions. Before mechanisms can be studied, it is necessary to ascertain how localized growth compares to broader trends.

The success of this work was due largely to the cooperative work with ADFG and the sport fishing community in Homer. This type of sampling is viable in other ports where fish are filleted, particularly in areas with established port sampling programs through entities such as ADFG and IPHC. In addition to size-at-age, it is viable to study other aspects of halibut biology through port sampling. We also studied diet, disease and stable isotopes of our port-sampled fish. All of these factors are being analyzed in relation to size-at-age to assess potential mechanisms for the region wide reduction in exploitable biomass. Through increased knowledge

of localized differences in size-at-age and mechanisms, managers can make more informed decisions to reduce economic stressors for charter and commercial fleets and to ensure the sustainability of the fisheries.

CHAPTER 2

ASSESSMENT OF PACIFIC HALIBUT DIET USING CARBON AND NITROGEN STABLE ISOTOPE VALUES

2.1 INTRODUCTION

Marine ecosystems are vast and dynamic, and seemingly small changes may have large impacts on species, populations or areas. The Gulf of Alaska plays host to organisms ranging from amphipods to corals to teleosts to marine mammals. This highly productive area is also economically essential to the state of Alaska due to its expansive fishing grounds (Gaichas *et al.* 2011). For example, while the cause of the 1992 decline in herring populations in Prince William Sound is still disputed (Pearson *et al.* 1999, 2012), it led to both ecosystem stresses due to the reduction of forage fish and economic stresses to herring fishers (Pearson *et al.* 1999). Recent declining trends in Pacific halibut (*Hippoglossus stenolepis*) size-at-age are concerning and unexplained and may be related to changes in the marine ecosystem.

The International Pacific Halibut Commission (IPHC) estimates that in 2013 the total removals of halibut were about 46 million pounds (IPHC 2014). US commercial fisheries (97.7% in Alaska) accounted for about 30 million pounds of removals, valued at $116.9 million (NMFS 2014). Commercial, sport and personal use landings of Pacific halibut in southcentral Alaska (IPHC regulatory area 3A) accounted for about 52.7% of the total landings from Alaskan halibut fisheries (IPHC 2014). Despite stable or increasing levels of halibut biomass in 3A over the past two decades, fish size-at-age has been declining (IPHC 2006a). The result is a shrinking proportion of fish in the exploitable size range (≥81 cm), leading to reduced harvest allocations even when fish abundance is high (See Chapter 1). The causes of declining halibut size-at-age are unknown.

The influence of halibut diet on their size-at-age is poorly understood. Generally, fish growth is governed by bioenergetic processes where, energy for growth = energy ingested – (energy used for maintenance + energy lost to the environment + energy used for reproduction). Growth is defined as an individual fish's increase in length or weight with time (NOAA 2006b), while size-at-age is the length or weight of a fish at a particular age (NOAA 2006b) and is used to back calculate growth rates. Factors influencing halibut size-at-age may include changes in abundance, recruitment, fishing pressure, climate, prey availability, disease and abundance of other species (Lee 1912, Clark *et al*. 1999, Clark and Hare 2002, Conover and Munch 2002, Gaichas *et al.* 2011, Orlov *et al*. 2011, Stephen *et al.* 2011, Kocan et al. 2010, IPHC 2013b). Clark and Hare (2002) thought that increased abundance of halibut might be causing decreased size-at-age via competition for food. In addition, halibut forage varies with depth, geographical location and sex (Orlov *et al*. 2011) and thus may be an important driver of size-at-age differences (IPHC 2008). Declines in prey availability or quality due to environmental changes, or competition with other species (e.g. Arrowtooth flounder, *Atheresthes stomias*) can result in reduced size-at-age (Gaichas *et al.* 2011, IPHC 2013b).

In order to assess the impacts of diet on size-at-age, it is first necessary to better define halibut diet. Halibut diets have been described only generally using gut content analyses. Known prey include fishes, crustaceans, mollusks, echinoderms, cephalopods, marine worms, kelp and fisheries offal (IPHC 1986, 2000b, 2008, Orlov and Moukhametov 2007, Roseneau and Byrd 2000). The 1999 and 2001 Alaska Fisheries Science Center (AFSC) surveys of commercially important groundfishes in the Gulf of Alaska found that walleye pollock (*Theragra chalcogramma*), Pacific sand lance (*Ammodytes hexapterus*) and capelin (*Mallotus villosus*)

contributed the most, by weight, to halibut diet. Hermit crabs (*Pagurus sp.)*, by weight of invertebrate species, were also a top contributor (NOAA 2006A). In Cook Inlet, juvenile and adult halibut eat capelin, sand lance, flatfish, sculpin (*Cottidae, sp.)*, Pacific cod (*Gadus macrocephalus)*, crabs, shrimp, squid, octopuses, and mollusks (Roseneau and Byrd 2000). Anecdotal information from fishers in the Port of Homer suggests that halibut are now consuming more crustaceans (especially crabs) and less forage fish. Because diets vary widely across regions, it is important to study them locally, especially when looking for correlations between diet and other factors such as size-at-age.

Since the formation of the IPHC in 1923, substantial and ongoing research has focused on halibut population dynamics, but relatively few studies have investigated diet and none have explored the impacts of diet on size-at-age. Most fieldwork related to halibut biology is done during IPHC stock assessment cruises. The IPHC conducts annual halibut abundance and size distribution assessments. Samples are collected in the United States and Canada from Oregon to the Bering Sea at regular stations (total 1,274 stations in 2012) on a 10x10 nautical mile grid in depths ranging from 35 to 500 meters using standardized setline surveys. Owing to the large geographical survey area the assessment requires simultaneous sampling with many vessels; for example, the 2012 survey used a total of 686 charter days on 10 vessels. During the survey, 55,000 halibut were landed and 17,900 otoliths were collected for size-at-age analysis (IPHC 2013d). Presently, stomach content data are not collected on the surveys. In order to relate size-at-age to diet, stomach contents must be associated with individual fish. Due to the cost and scope of projects that are included in the surveys, additional large-scale sampling to examine stomach contents is not practical and consistency among crews would be problematic. Port

sampling is used by the IPHC and other management agencies (e.g. Alaska Department of Fish and Game (ADFG)) to assess size-at-age in commercial and sport catches of halibut and other fishes that are actually being landed (e.g. Scheirer *et al.* 2004, IPHC 2006a). Port sampling can be useful in studying biological concepts that are impractical to study on surveys. For example, in addition to length, age and sex data, ADFG, US Geological Survey (USGS), APU, IPHC and the Washington Animal Diagnostics and Disease Lab (WADDL) used port sampling during the sampling season in 2011 and 2012 to assess *Ichthyophonus* and Mushy Flesh Syndrome in halibut and other groundfishes (ADFG 2012, 2013).

Stomach content analyses can provide definitive species-level prey samples but are of limited use for diet-growth studies. Stomach contents only provide a recent "snapshot" of what the fish has consumed, and sometimes provide no information due to regurgitation during capture. Stomach content analyses are also potentially biased due to rapid (hrs to days) and variable digestion rates (Berens and Murie 2008). For example, crustacean exoskeletons typically remain in the stomach longer than more easily digestible soft tissues, such as fish flesh (Hopkins and Larson 1990). This is why many stomach content analyses rely on identification of bones and otoliths (Bowen 1983, Whitfield and Blaber 1978).

Stable isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) ratios have been used to successfully evaluate the diets of terrestrial and marine species including teleost fishes (for examples see Fry 1988, Benstead *et al.* 2006, Fernandez *et al.* 2011). In aquatic environments, $\delta^{13}C$ is reflective of benthic ($\delta^{13}C$ depleted) and pelagic ($\delta^{13}C$ enriched) prey sources (Sherwood and Rose 2005, Sherwood *et al.* 2007), while $\delta^{15}N$ is reflective of trophic level (higher level = more enriched $\delta^{15}N$; Fry 1988).

When a fish consumes prey, its tissues retain the stable isotope (e.g. $\delta^{13}C$ and $\delta^{15}N$) ratios of prey in proportion to assimilation (not consumption) until the tissues turnover (Phillips 2012, Marsh *et al.* 2012). The rate at which prey is incorporated into the predator's tissues is termed the *assimilation rate*. The *turnover rate* is the time it takes for a given consumer's tissue to reflect the isotope composition of food resources and is the result of both tissue growth and tissue replacement; this varies by tissue type (Madigan *et al.* 2012). Inert tissues like otolith do not turnover. Ultimately the ratios of $\delta^{13}C$ and $\delta^{15}N$ in a fish's tissues (e.g. muscle or otolith) reflect some past proportional contribution of prey items to that fish's diet determined by the turnover rate (Fernandez *et al* 2011). The IPHC (2003) and Marsh *et al.* (2012) used $\delta^{13}C$ and $\delta^{15}N$ ratios to evaluate halibut trophic level, but no studies have applied stable isotope analyses to assess proportional dietary contributions of halibut prey. This is probably because the stable isotope ratios of most halibut prey were unknown.

Homer, AK, is located on Kachemak Bay in Lower Cook Inlet and supports the largest Pacific halibut sport fishery in the United States (Meyer and Powers 2013; Figure 1). Sport fishers and charter fishing operations typically fillet their catch at fish processing stations or businesses located at the port (Figure 1.1). Once filleted, the carcasses are discarded in municipal dumpsters, and ultimately are ground and dumped at sea. To assess fishing effort and the size, age, and sex of sport-caught halibut, staff from the Alaska Department of Fish and Game (ADFG), Division of Sport Fish, Groundfish Research Program interview fishers and sample the catch at these stations. In May 2012, I worked cooperatively with ADFG to expand port-sampling to include diet (stomach contents and stable isotopes, $\delta^{13}C$ $\delta^{15}N$, in flesh).

The aim of this chapter is to describe the diet of halibut in Lower Cook Inlet and Kachemak Bay and to 1) determine whether the mean isotopic values of halibut varied by size, age, sex, location and sample date, and 2) assess trends in isotopic values relative to size-at-age to explore whether diet may be contributing to recent changes in size-at-age. We found that halibut isotope signatures differed significantly among all assessed factors indicating that diet varies with sex, size, age, spatially and temporally. Further, fast growing fish appear to have a more benthic diet and feed at a higher trophic level.

2.2 METHODS

Field Sampling

Fork length and mouth gape, sex and stomach contents, both sagittal otolith, and flesh tissue samples were collected from filleted halibut carcasses in the port of Homer prior to disposal. I used a size-stratified design aimed at sampling at least 30 male and 30 female halibut in as many 10-centimeter fork length size bins as possible (< 39 cm, 40 – 49 cm, 50 – 59 cm, etc.). Fork length was measured for each fish to the nearest cm, and a visual evaluation of gonads was used to determine the sex following the 2012 Field Procedure Manual for the Southcentral Alaska Halibut and Groundfish Harvest Assessment Program of ADFG. Finally, catch location (ADFG Statistical Area) was requested of the charter captain or private fisher.

Intact prey samples were collected from halibut stomachs and frozen for stable isotope analyses. Up to 20 samples of each prey item were collected based on availability in stomach contents. Halibut muscle tissue (with skin removed) was taken from the proximal dorsal portion of the blind side of the halibut, just above the cheek and frozen for stable isotope analyses.

Prey Analysis

The stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ of the prey items were determined. Due to the extremely low pH of their stomachs, halibut can fully digest both hard and soft tissues of most prey. Therefore, we analyzed whole prey samples collected from halibut stomachs. Samples were oven-dried (42°C for 72 to 168 hrs) and ground to a fine powder using a mortar and pestle or small high-speed food processor. Samples were analyzed at the University of Wyoming's Stable Isotope Facility using a Carlo Erba 1110 Elemental Analyzer[1] coupled to a Thermo Delta Plus XP IRMS[2]. Long-term analyses of quality control standards at this facility have yielded a precision of 0.3 per mil for $\delta^{13}C$ and 0.4 per mil for $\delta^{15}N$. Vienna Pee Dee Belemnite and air were used as standards, respectively, for all analyses.

Prey stable isotope values were established based on the mean and standard error of each prey's $\delta^{13}C$ and $\delta^{15}N$. Prey values were pooled into four categories including teleosts, crustaceans, cephalopods, and amphipods following McIntyre *et al*. (2006). Species with less than 1% occurrence in stomachs that were not included in these broad categories were omitted from further isotope analysis. MANOVAs were used to assess $\delta^{13}C$ and $\delta^{15}N$ overlap between the four prey groups and Tukey post-hoc tests were used to identify pairwise differences (Zar 1999).

Halibut Analysis

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in halibut muscle tissue were analyzed to determine the diet proportions of individual fish and groups of fish (e.g. by size, sex, age, location and sample

[1] Carlo Erba Reagents, CE Instruments, ThermoQuest Italia S.p.A. Milan, Italy
[2] Thermo Finnigan, Bremen, German

date). Samples were oven-dried (42°C for 72 hrs) and ground to a fine powder using a mortar and pestle or small high-speed food processor. Samples were analyzed at the University of Alaska Anchorage Stable Isotope Facility using a Costech ECS 4010 elemental analyzer[3] coupled to a Thermo-Finnigan Delta V Advantage mass spectrometer[4] (long-term analyses of quality control standards at this facility have yielded a precision of 0.2 per mil for $\delta^{13}C$ and $\delta^{15}N$) or at University of Wyoming Stable Isotope Facility described above.

Stable isotope values were established for groups of fish based on the mean and standard error of their $\delta^{13}C$ and $\delta^{15}N$. MANOVAs were used to look for $\delta^{13}C$ and $\delta^{15}N$ overlap between groups. Tukey post-hoc tests were used to identify pairwise differences.

Linear regressions were used to examine the relationship between age - $\delta^{13}C$ and age - $\delta^{15}N$ of fish within a 10-cm size bin. T-tests were then used to determine if there were significant differences in $\delta^{13}C$ and $\delta^{15}N$ between the younger and older of the fish with approximately 50% of the fish from a size bin in each group. An additional t-test was used to determine if there was a significant difference in the length of the younger and older fish.

Bayesian mixing models were used to estimate the contribution of prey to halibut diets by fast, moderate and slow growing fish for the two size bins with the most samples: 80 cm females (n = 93) and 70 cm males (n = 87). Relative proportions of prey assimilated by each halibut were determined using a Bayesian mixing model based upon a Gaussian likelihood with a mixture dirichlet-distributed prior on the mean,

[3] Costech Analytical, Valencia, CA., USA
[4] Thermo Fisher Scientific Inc, Waltham, MA., USA

$\delta^{13}C_{consumer} = f_{C1}\delta^{13}C_1 + f_{C2}\delta^{13}C_2 + \ldots + f_{Cn}\delta^{13}C_n$

$\delta^{15}N_{consumer} = f_{N1}\delta^{15}N_1 + f_{N2}\delta^{15}N_2 + \ldots + f_{Nn}\delta^{15}N_n$

$f_1 + f_2 + \ldots + f_n = 1$

where $\delta^{13}C_i = \delta^{13}C$ value of prey i, etc. weighted by fractionation value f_{Ci} and $\delta^{15}N_i = \delta^{15}N_i$ value of prey i, etc. weighted by fractionation value f_{Ni}. Models were fitted using the software package MIX Stable Isotope Analysis in R (MixSIAR, Stock *et al*. 2013). Fractionation values of 1.0 ± 0.4 per mil for $\delta^{13}C$ and 3.4 ± 1.0 per mil for $\delta^{15}N$ were applied to the models (Dennard *et al.* 2009). Models were run using the "normal" Markov chain Monte Carlo (length = 100,000, burn in = 50,000, thinning = 50, chains = 3) with both process and residual error. Halibut growth rate and individual variability were considered random effects in the analyses.

2.3 RESULTS

Summary of field sampling

We sampled 429 fish in 2012 and 316 in 2013 for a total of 745. Of these, 588 were sampled for stable isotopes in flesh. Females ranged from 24 to 200 cm fork length and 2 to 25 years (Figures 1.4, 1.5). Target sample size (n = 30) was achieved for females in the 60, 70, 80, 90, 100, and 110 size bins. Male fish ranged from 41 to 107 cm fork length and 4 to 29 years (Figures 1.4, 1.5). Target samples size for males was reached in the 60, 70 and 80 cm size bins.

Stable Isotope Ratios ($\delta^{13}C$ and $\delta^{15}N$) of Halibut Prey

Prey had a wide range of stable isotope values of both $\delta^{13}C$ (Min = -22.0, Max = -5.9) and $\delta^{15}N$ (Min = 6.5, Max = 19.7). Values for fishes ranged from -22.0 to -16.6 for $\delta^{13}C$ (mean = -19.1) and 10.3 to 14.7 for $\delta^{15}N$ (mean = 12.3; Figure 2.1). Values for crabs ranged from -19.4 to -11.1

for $\delta^{13}C$ (mean = -15.2) and 6.8 to 13.8 for $\delta^{15}N$ (mean = 11.2; Figure 2.2). Combine $\delta^{13}C$ and $\delta^{15}N$ values were significantly different for teleosts, crustaceans, cephalopods, and amphipods (MANOVA, $F(3, 179) = 34.76$, $p < 0.001$). There were significant differences in $\delta^{13}C$ between teleosts and crustaceans, cephalopods and teleosts, and amphipods and teleosts. There were significant differences in $\delta^{15}N$ between teleosts and crustaceans, cephalopods and crustaceans, amphipods and crustaceans, amphipods and teleosts, and amphipods and cephalopods (Figure 2.3, see appendix for post hoc results).

Stable Isotope Ratios ($\delta^{13}C$ and $\delta^{15}N$) of Halibut Muscle

Halibut muscle had a wide range of stable isotope values of both $\delta^{13}C$ (Min = -18.7, Max = -14.7, Mean = -17.0) and $\delta^{15}N$ (Min = 13.4, Max = 19.6, Mean = 15.6; Figure 2.4). There were significant differences in $\delta^{13}C$ and $\delta^{15}N$ ratios for all factors: sex, location, sample date, age and length (MANOVAs, $p<0.01$; table 2.1, see appendix for figures and post hoc results). Difference in $\delta^{15}N$ between sexes suggests that on average females are at a trophic level that is 29% higher than males and that the females have a slightly more benthic diet. Generally, fish caught in nearby locations were more similar than fish caught further apart. Fish from Kachemak Bay appear to have a more benthic diet than fish in the Gulf of Alaska, while diet of fish from Cook Inlet is more variable. Fish sampled during the same year were relatively similar, while fish sampled in different years were much more different. Fish sampled in 2012 were slightly more enriched in both $\delta^{13}C$ and $\delta^{15}N$ indicating a more benthic diet and a higher trophic level. The youngest fish were more depleted in $\delta^{15}N$, indicating that they are feeding at a lower trophic level, while the oldest fish were more depleted in $\delta^{13}C$, indicating a more pelagic diet. The

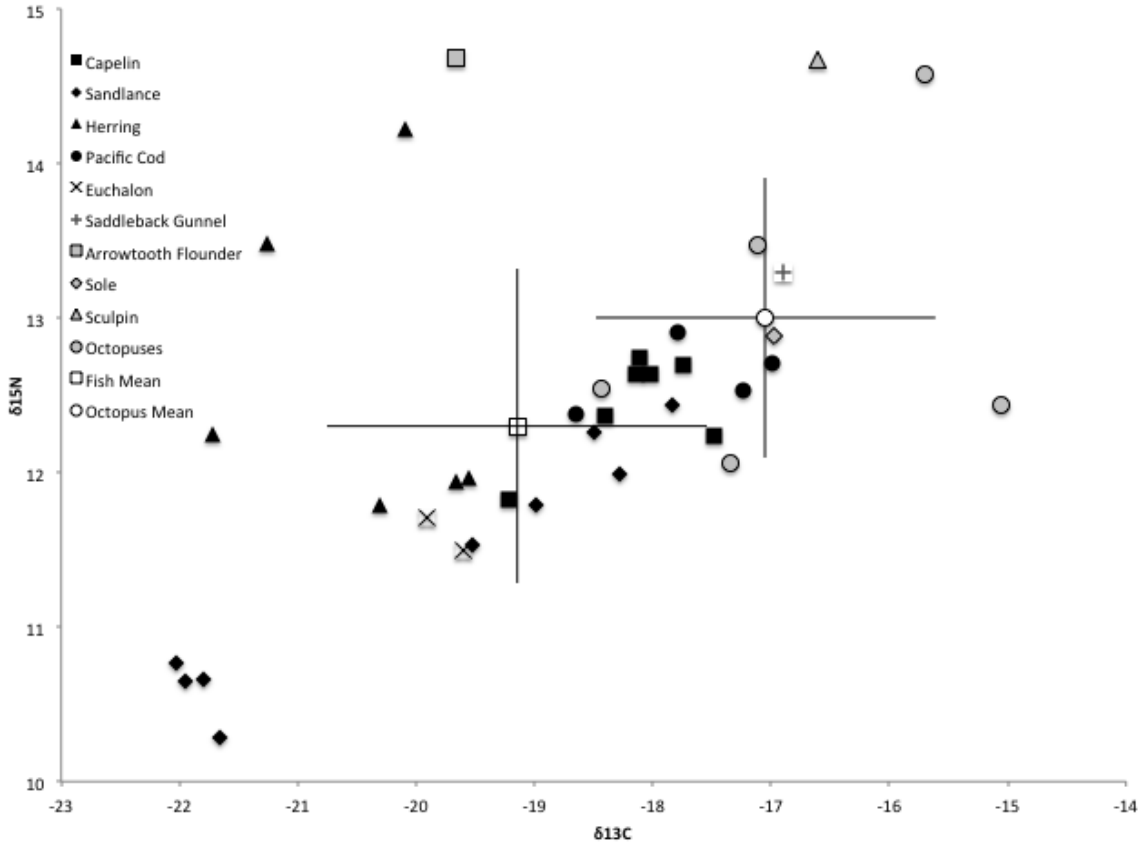

Figure 2.1. All values of $\delta^{13}C$ and $\delta^{15}N$ for sampled teleosts and cephalopods. Means are given ± 1 standard error. Results within the same species were often variable.

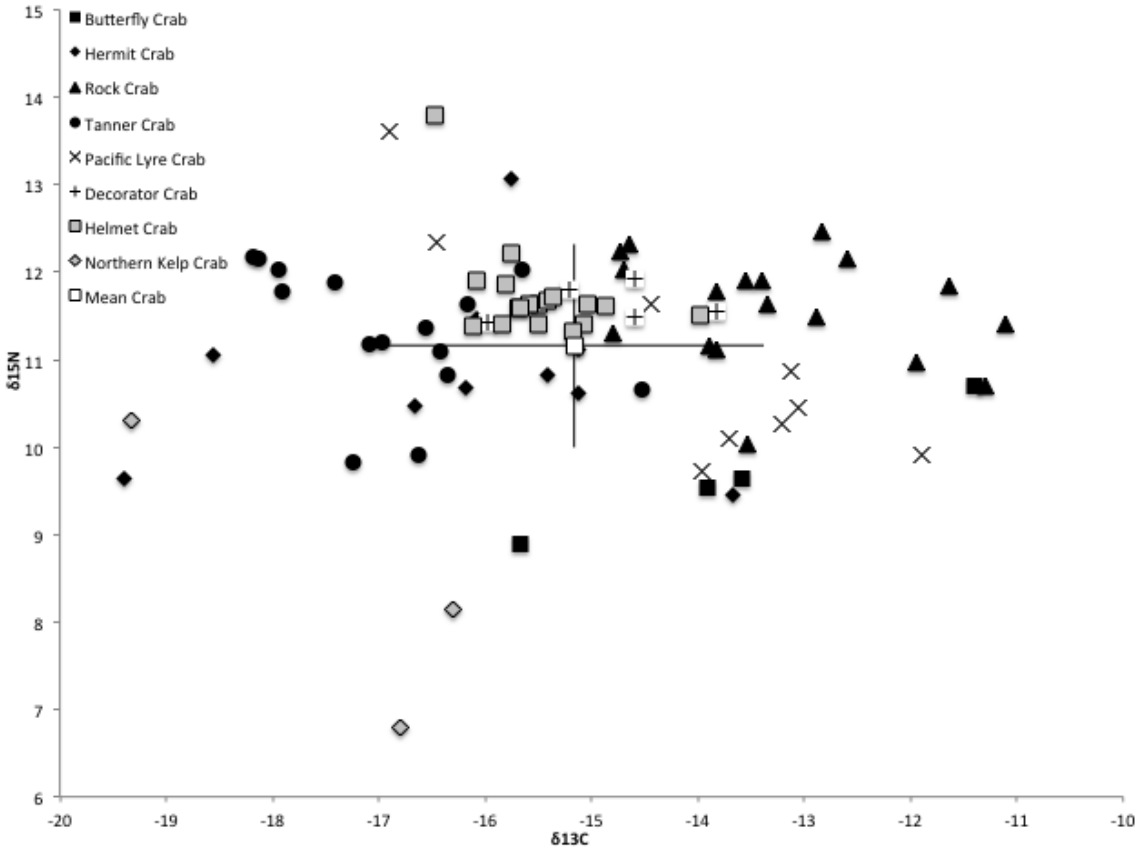

Figure 2.2. All values of $\delta^{13}C$ and $\delta^{15}N$ for sampled crustaceans. Means are given ± 1 standard error. Results within the same species were often variable.

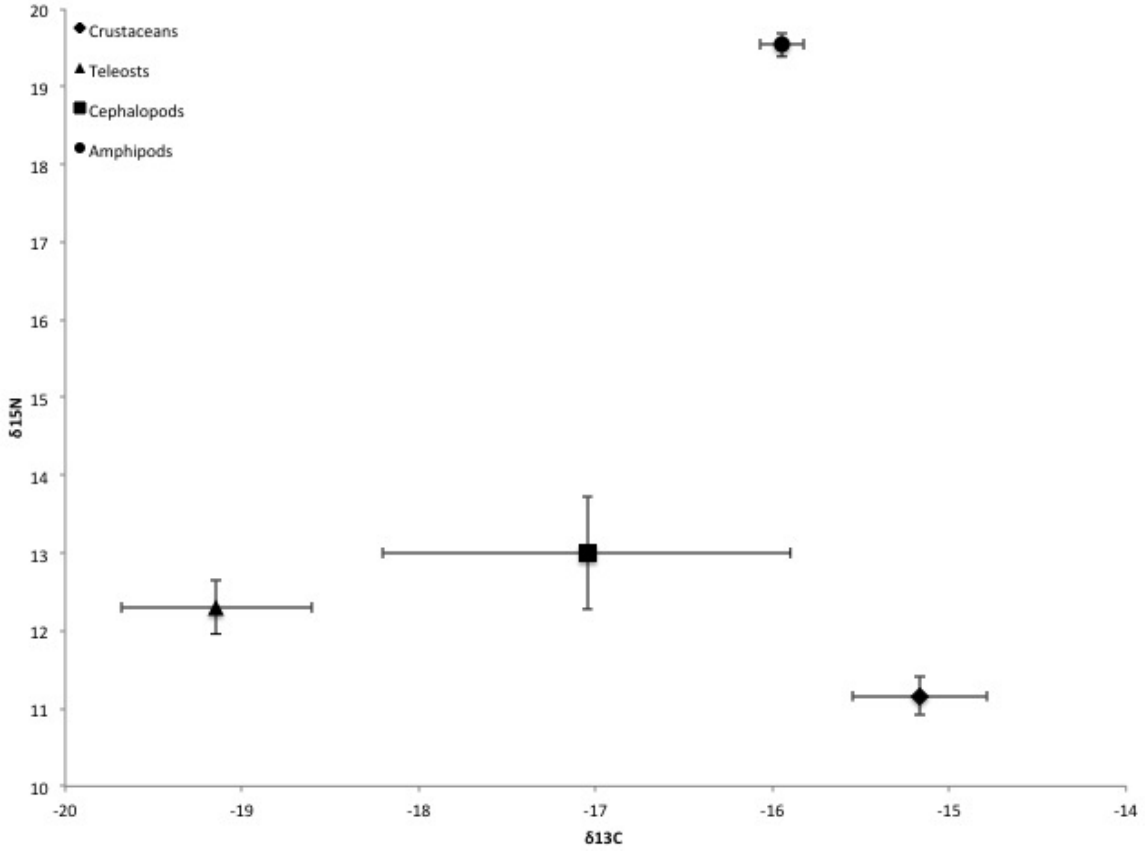

Figure 2.3. Mean values of $\delta^{13}C$ and $\delta^{15}N$ for crustaceans, teleosts, cephalopods and amphipods with 95% confidence intervals. $\delta^{13}C$ and $\delta^{15}N$ values were significantly different for all groups.

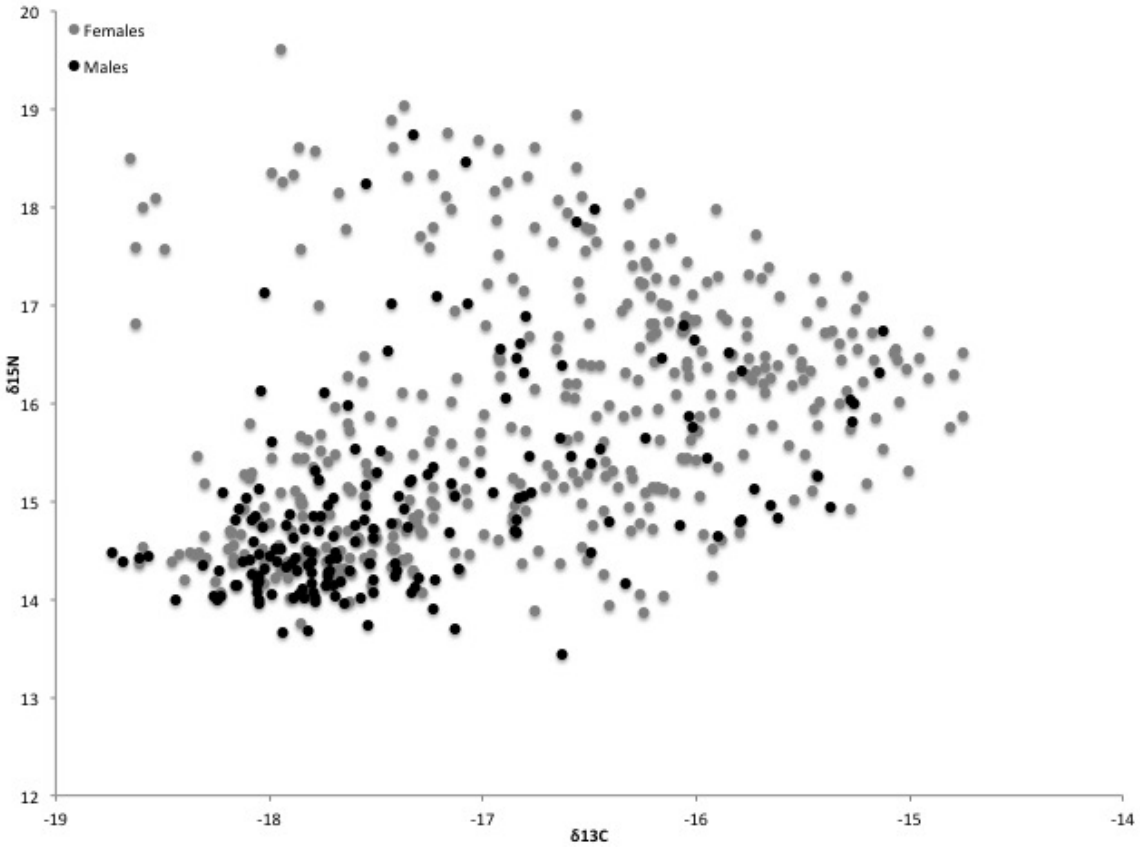

Figure 2.4. $\delta^{13}C$ and $\delta^{15}N$ values for all sampled halibut. Females tended to have a wider range of values.

Table 2.1. MANOVA results for comparing $\delta^{13}C$ and $\delta^{15}N$ with sex and by sex comparing $\delta^{13}C$ and $\delta^{15}N$ with size, age, location and sampling date.

	Degrees of Freedom	F	P-value
Sex	1, 578	48.8	<0.001
Female Size	17, 381	3.8	<0.001
Male Size	6, 173	3.4	<0.001
Female Age	21, 365	1.6	<0.01
Male Age	23, 155	2.1	<0.001
Female Location	16, 259	5.3	<0.001
Male Location	11, 132	2.4	<0.001
Female Date	8, 389	6.8	<0.001
Male Date	8, 170	2.6	<0.001

smallest fish (< 60 cm) were more depleted in $\delta^{15}N$ indicating a lower trophic level, while larger fish were more enriched in $\delta^{15}N$, with little difference after 100cm. Small (< 60 cm) and large (> 100 cm) had similar and low $\delta^{13}C$ signatures, indicating a benthic diet, while medium size fish had a more pelagic diet.

Stable Isotope Ratios ($\delta^{13}C$ and $\delta^{15}N$) of Halibut Muscle and Size-At-Age

For female halibut, linear regressions of $\delta^{13}C$ and $\delta^{15}N$ on age within 10-cm size bins had variable results (Table 2.2). Of the 8 size bins (50 – 120 cm) analyzed, 3 of the younger groups were significantly more enriched in $\delta^{13}C$ and $\delta^{15}N$ (70, 80, 90) indicating a more benthic diet from a higher trophic level (Table 2.3). In the size bin with the most fish, 80 – 89 cm, age explained 86% of the variation in mean $\delta^{13}C$ and 71% of the variation in mean $\delta^{15}N$ (Figure 2.5). For male halibut, linear regressions of $\delta^{13}C$ and $\delta^{15}N$ on age within 10-cm size bins also had variable results (Table 2.4). Of the 5 size bins (50 – 90 cm) that were analyzed, 2 of the younger groups were significantly more enriched in $\delta^{13}C$ and $\delta^{15}N$ (70 and 80 cm) indicating a more benthic diet from a higher trophic level (Table 2.5). In the 70 – 79 cm size bin, age explained 59% of the variation in mean $\delta^{13}C$ and 67% of the variation in mean $\delta^{15}N$ (Figure 2.6).

Bayesian implementations of mixing models indicated a wide range of possible dietary proportions (see appendix). In the 80 cm females, results show that there may be a bias for octopuses for younger, fast growers and a bias for fishes for older, slow growers (Figure 2.7 and 2.8). In the 70 cm males, results show that the prey field for younger, fast growers is much more variable than the prey field for slow growers (Figure 2.9 and 2.10).

Table 2.2. Linear regression equations and R^2 values of $\delta^{13}C$ and $\delta^{15}N$ on age by size bin for female halibut. Strength of the regression is highly variable depending on the size bin. The bin with the largest sample size (80 cm) had the strongest regressions.

Length	δ13C equation	δ13C r-squared	δ15N equation	δ15N r-squared
50	y = -0.2945x - 14.884	0.308	y = 0.1039x + 14.331	0.095
60	y = -0.2823x - 15.061	0.860	y = -0.1159x + 16.072	0.137
70	y = -0.1446x - 15.794	0.459	y = -0.0876x + 15.97	0.124
80	y = -0.2289x - 14.651	0.865	y = -0.1917x + 17.477	0.712
90	y = -0.0961x - 15.779	0.362	y = -0.0411x + 16.232	0.138
100	y = -0.2007x - 14.56	0.427	y = -0.0156x + 16.313	0.006
110	y = -0.0633x - 15.34	0.088	y = -0.0138x + 16.452	0.010
120	y = -0.0284x - 16.282	0.016	y = 0.0188x + 16.547	0.008

Table 2.3. T-test results for female halibut between older and younger groups of fish within size bins. There were 4 size bins with significant differences for $\delta^{13}C$, 3 for $\delta^{15}N$ and 2 for length.

Length	Younger N	Younger range	Older N	Older Range	Younger mean δ13C	Older mean δ13C	δ13C p-value	Younger mean δ15N	Older Mean δ15N	δ15N p-value
50	12	4 to 5	4	6 to 8	-16.330	-16.912	0.1846	14.818	15.086	0.3895
60	18	5 to 6	10	7 to 11	-16.804	-17.191	0.2188	15.034	15.844	0.0834
70	33	6 to 8	39	9 to 14	-16.781	-17.531	0.0001	15.954	14.776	0.0004
80	48	6 to 9	45	10 to 17	-16.652	-17.360	0.0002	16.311	15.022	0.0000
90	29	5 to 11	24	12 to 25	-16.311	-17.490	0.0000	16.357	15.402	0.0000
100	20	6 to 11	16	12 to 16	-16.196	-16.957	0.0323	16.518	16.118	0.2638
110	16	8 to 11	9	12 to 16	-16.091	-16.247	0.6754	16.572	16.169	0.3053
120	13	8 to 11	14	12 to 16	-16.117	-16.626	0.1297	16.632	16.944	0.3602

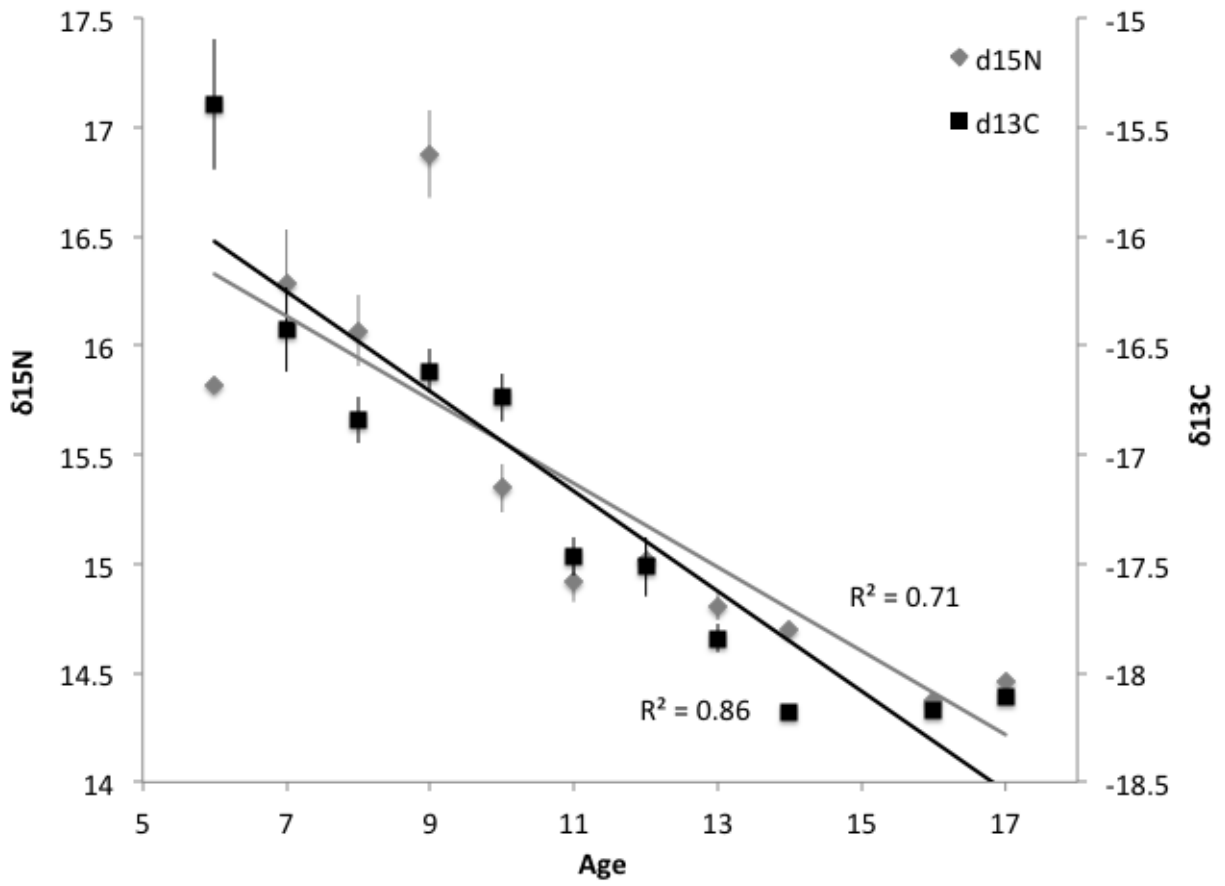

Figure 2.5. Mean $\delta^{13}C$ and $\delta^{15}N$ values ± 1 standard error by age for 80 – 89 cm female halibut. Age explained 86% of the variation in mean $\delta^{13}C$ and 71% of the variation in mean $\delta^{15}N$.

Table 2.4. Linear regression equations and R^2 values of $\delta^{13}C$ and $\delta^{15}N$ on age by size bin for male halibut. Strength of the regression is highly variable depending on the size bin. The bin with the largest sample size (70 cm) had the strongest regressions.

Length	δ13C equation	δ13C r-squared	δ15N equation	δ15N r-squared
50	y = 0.1017x − 16.069	0.062	y = 0.2887x + 13.151	0.628
60	y = 0.1133x − 15.992	0.196	y = 0.034x + 15.379	0.016
70	y = 0.1219x − 16.056	0.594	y = 0.1802x + 17.131	0.668
80	y = 0.0685x − 16.24	0.477	y = 0.1124x + 17.02	0.448
90	y = 0.1388x − 14.702	0.669	y = 0.0519x + 16.147	0.255

Table 2.5. T-test results for male halibut between older and younger groups of fish within size bins. There were 3 size bins with significant differences for $\delta^{13}C$, 2 for $\delta^{15}N$ and 3 for length.

Length	Younger N	Younger Range	Older N	Older Range	Younger mean δ13C	Older mean δ13C	δ13C p-value	Younger mean δ15N	Older Mean δ15N	δ15N p-value
50	3	5	4	6 to 7	-16.806	-16.418	0.3395	14.723	14.795	0.9208
60	11	5 to 8	14	9 to 14	-16.856	-17.539	0.0458	15.158	14.819	0.3836
70	35	7 to 11	51	12 to 18	-17.333	-17.712	0.0137	15.125	14.568	0.0054
80	21	8 to 14	25	15 to 29	-16.873	-17.603	0.0011	15.930	14.601	0.0001
90	4	11 to 19	5	20 to 25	-16.809	-17.671	0.1301	15.347	15.045	0.4089

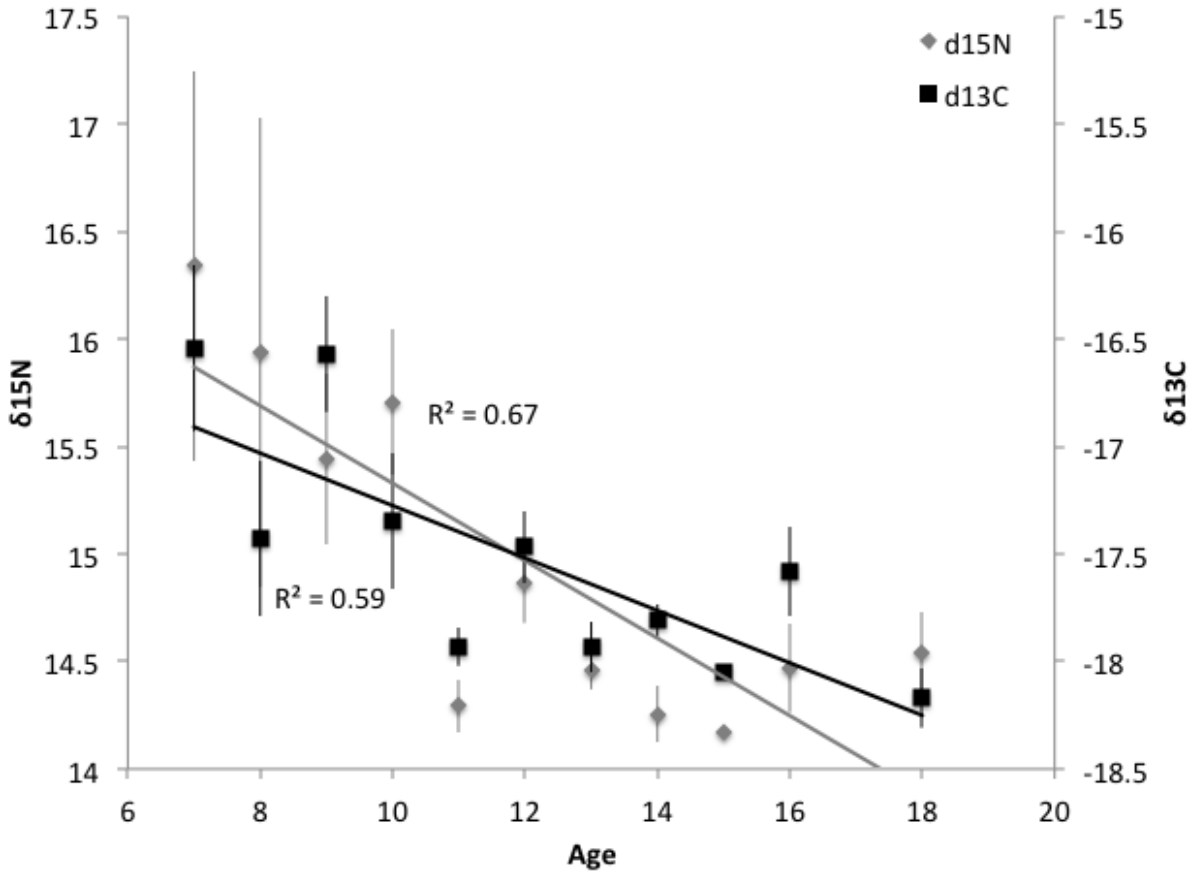

Figure 2.6. Mean $\delta^{13}C$ and $\delta^{15}N$ values ± 1 standard error by age for 70 – 79 cm male halibut. Age explained 59% of the variation in mean $\delta^{13}C$ and 67% of the variation in mean $\delta^{15}N$.

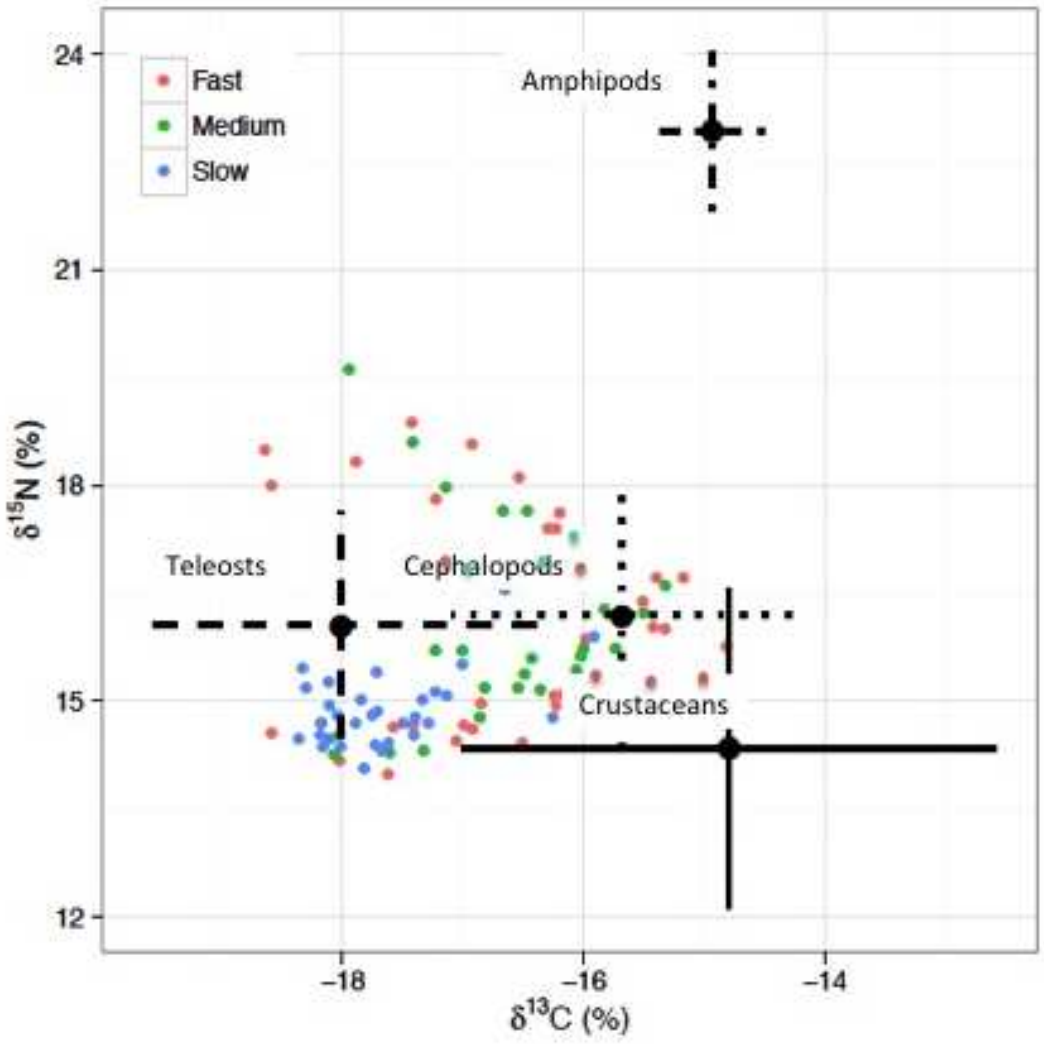

Figure 2.7. Isospace plot for 80-89 cm female halibut by growth speed, fast (6 – 8 years), medium (9 – 10 years) or slow (11 – 17 years).

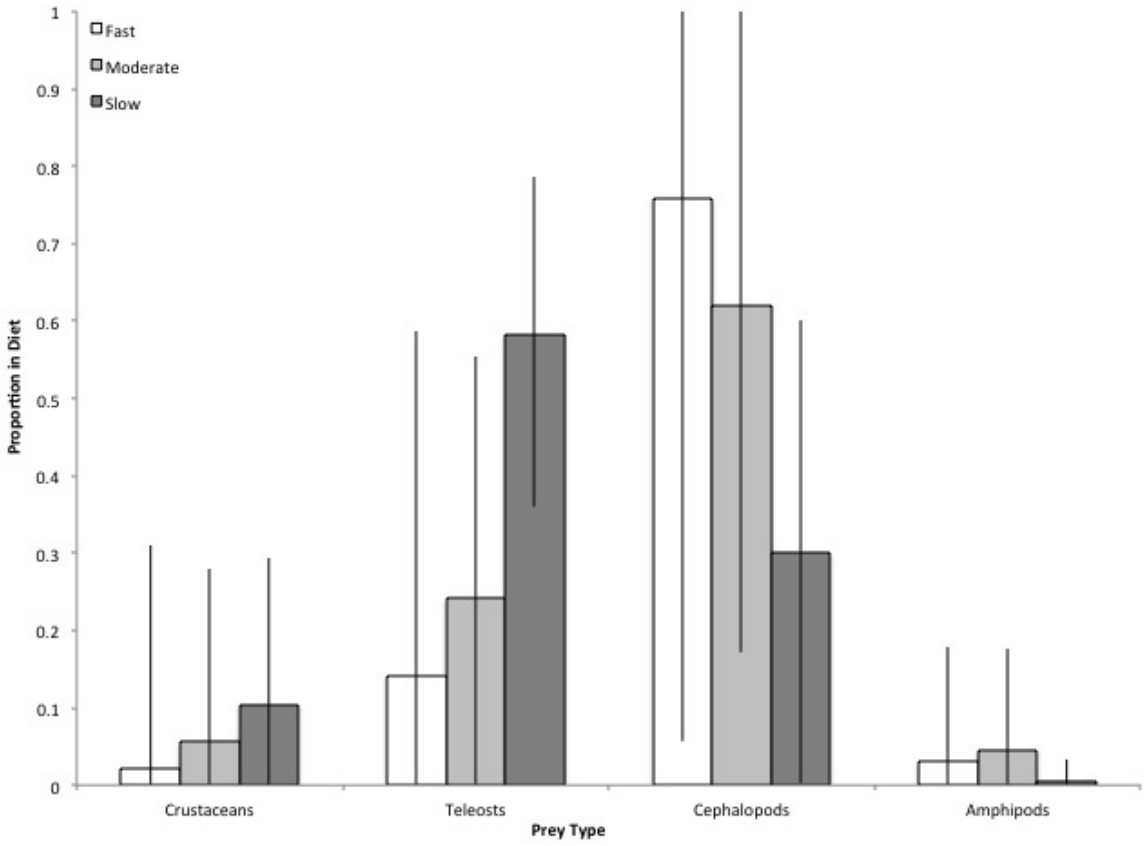

Figure 2.8. Proportional contribution of prey to 80- 89 cm female halibut diet by growth speed with 95% credibility intervals. Credibility intervals were much larger for faster growing fish.

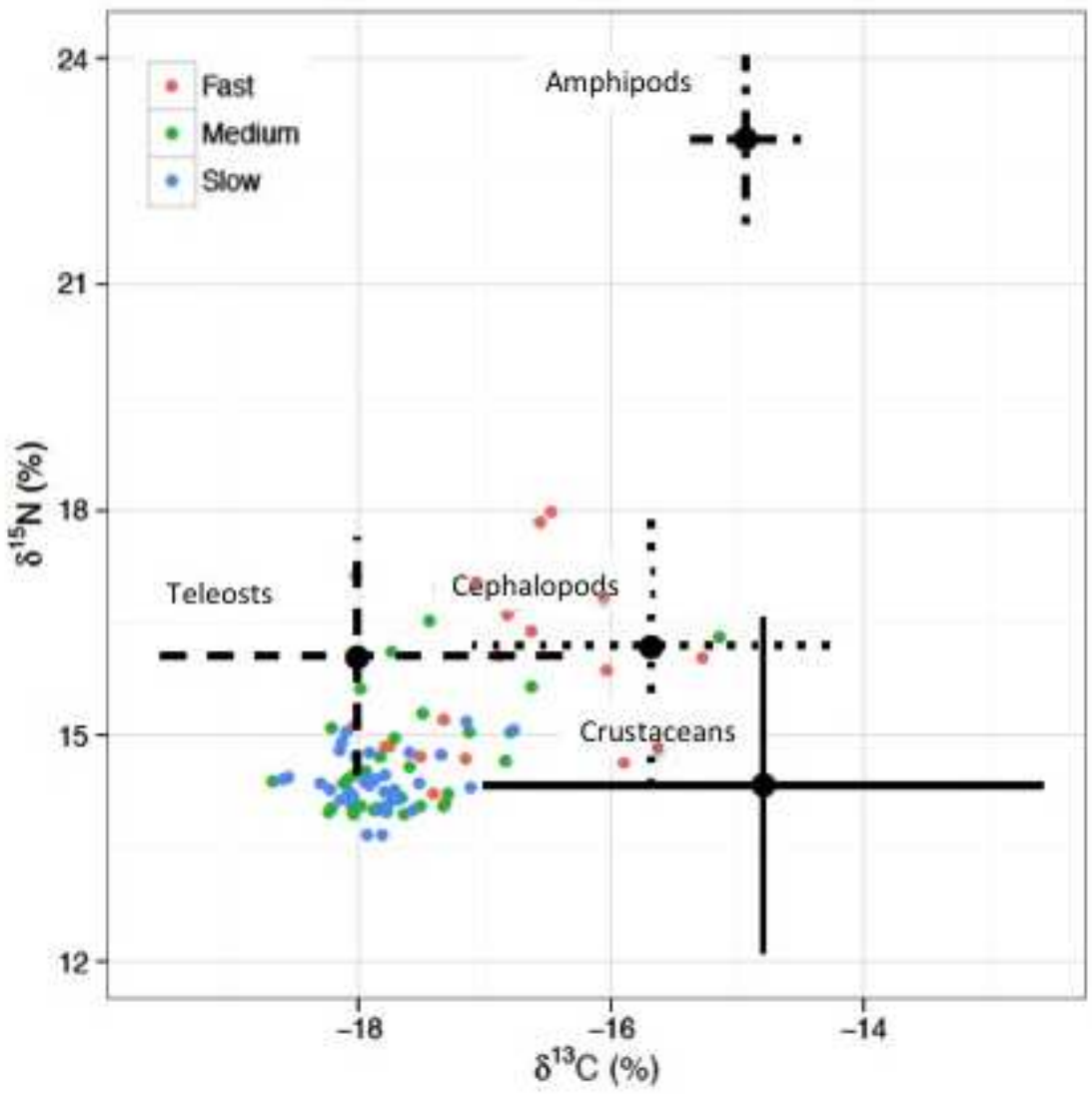

Figure 2.9. Isospace plot for 70-79 cm male halibut by growth speed, fast (7 – 10 years), medium (11 – 12 years) or slow (13 – 18 years).

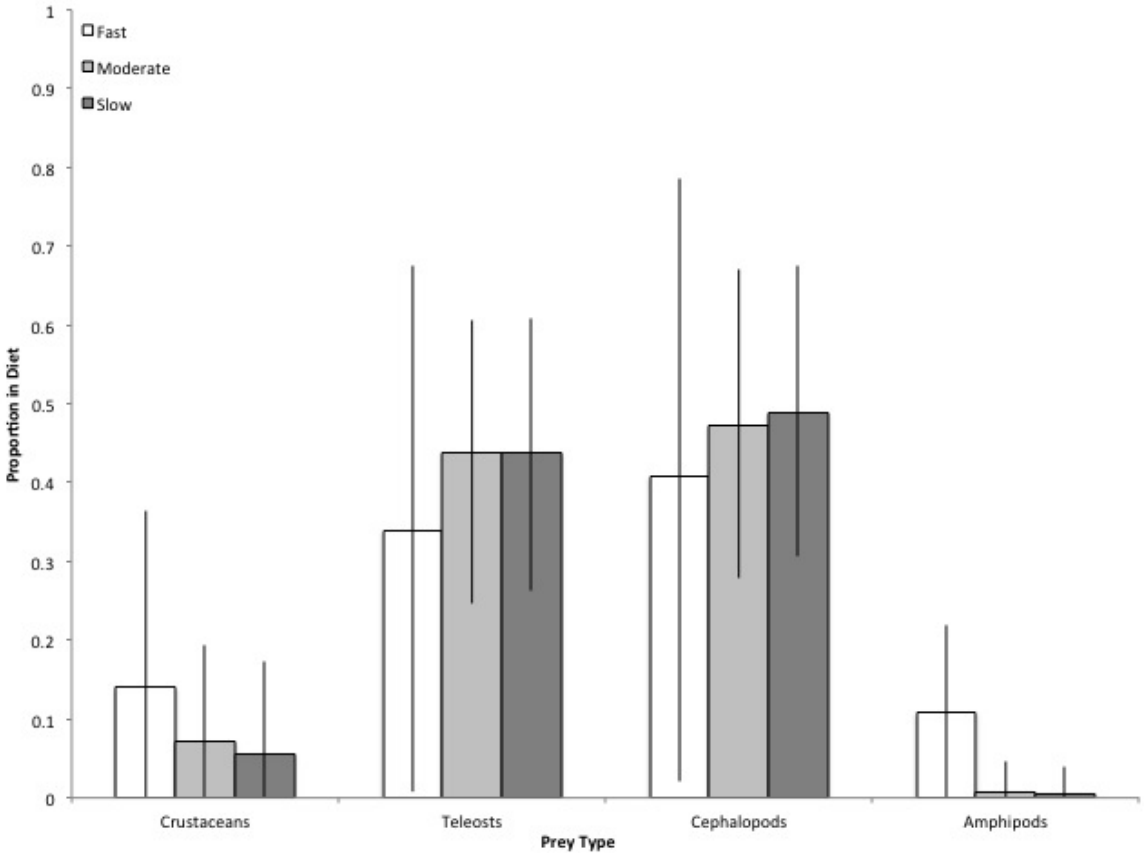

Figure 2.10. Proportional contribution of prey to 70- 79 cm male halibut diet by growth speed with 95% credibility intervals. Credibility intervals were much larger for faster growing fish.

2.4 DISCUSSION

Stable isotopes confirmed a wide breadth of prey assimilation. Ranges of both $\delta^{13}C$ and $\delta^{15}N$ in this study fell within previously published isotope values for halibut muscle (IPHC 2003, Marsh *et al.* 2012). In particular, $\delta^{13}C$ values were similar to the values obtained by Marsh *et al.* (2012) near Kodiak Island, also in 3A. Differences between male and female isotopic ratios are most likely explained by the sexual dimorphism in size. Because there is a very strong linear relationship between length and gape (length = 9.2*gape + 6.2, $R^2 = 0.86$, Figure 2.11), it follows that males are not capable of consuming prey from trophic levels as high as females. $\delta^{13}C$ values tended to be higher for halibut captured nearer to shore. Locational differences in $\delta^{13}C$ were expected, as $\delta^{13}C$ tends to vary with source, in particular benthic and pelagic environments (Hobson and Welsh 1992, Hobson *et al.* 1995, 2002). Similarities and differences in isotope values of locations were likely due to proximity of locations to each other. Temporal differences may be due to differences in prey availability from seasonal migrations and differences in fishing locations. Marsh *et al.* (2012) also detected significant temporal differences in halibut muscle $\delta^{13}C$ values. The lack of difference in $\delta^{13}C$ and $\delta^{15}N$ for most age groups was expected due to the high variability of size within each age class. The low number of significant differences in $\delta^{13}C$ and $\delta^{15}N$ among length classes was more surprising and may be related to low sample size in the very small and very large length classes. Differences in $\delta^{13}C$ and $\delta^{15}N$ relative to size-at-age were evaluated with linear regressions and t-tests. In several cases, in particular those with the most data, results showed significant differences in stable isotope composition of faster and slower growing fish. This demonstrates that stable isotopes may help identify the mechanisms causing slow growth.

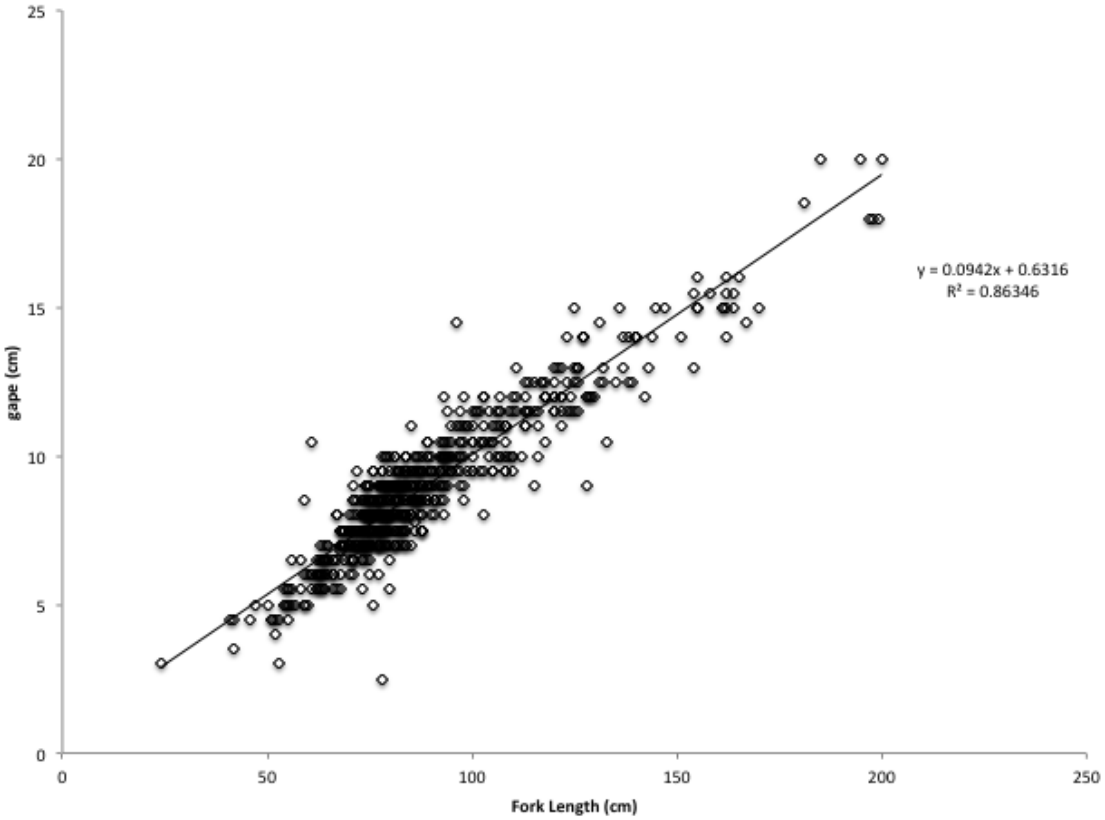

Figure 2.11. Scatter plot of halibut gape versus length showing a strong linear correlation between the two metrics. Outliers likely either had locked jaws (below average) or had ripped mouths (above average).

Stable isotope ratios of prey supported the hypothesis that groups of prey have unique isotopic signatures and may be useful in dietary analyses. Due to large amounts of isotopic overlap in similar species, prey were grouped for analysis. Larger sample size and greater control of sample location and size of individuals sampled may reduce the amount of error associated with prey isotope analyses. Prey were sampled from stomach contents of halibut, so actual location of ingestion is unknown and may encompass a wide area; in particular, this may impact $\delta^{13}C$ ratios. Further, controlling for size of prey would likely reduce the range of $\delta^{15}N$ ratios and should be considered in future analyses. Amphipods had a higher a $\delta^{15}N$ value higher than any other prey and higher than almost all halibut, suggesting that this group of species has a higher trophic level than halibut and the amphipods were most likely parasitic.

Bayesian mixing models were useful in identifying ranges of proportional contribution of prey to assimilated halibut tissue. However, several factors may have contributed to the utility of this tool. Isotopic signatures of prey appear to be significantly different from each other. Still, the central isospace position of octopus likely confounds the data. One possible solution to this problem would be to omit the amphipods from analysis, as Bayesian mixing models indicate that they contributed very little to the muscle. Discrimination factors also likely impacted the results. We were unable to find any laboratory studies identifying the discrimination factors of $\delta^{15}N$ and $\delta^{13}C$ for halibut muscle and therefore followed the methods of Dennard *et al.* (2009) for Greenland halibut. While discrimination factors for muscle are known to vary with fish species, most $\delta^{15}N$ discrimination factors from 3.0 – 3.4 per mil are considered unlikely to cause errors relative to trophic level (Sweeting *et al.* 2007). Like $\delta^{15}N$, the $\delta^{13}C$ discrimination factor that we used falls within the range of other field and laboratory studies; however, these are much more

variable (Caut *et al.* 2009). Several other factors may also impact discrimination factors including tissue type (Sweeting *et al.* 2007, Caut *et al.* 2009), prey (Sweeting *et al.* 2007, Caut *et al.* 2009), and growth of fish (Hesslein *et al.* 1993). Future studies using stable isotopes to assess Pacific halibut diet should therefore include laboratory components to determine appropriate discrimination factors.

Stable isotopes are a viable option for studying diet and other factors relating to halibut size-at-age. The best data will come from very large sample sets that can adequately sample all ages of fish within a size class. In addition to halibut muscle, which represents several months of fish diet, additional tissues should be used to assess diet over several days (blood) or diet over the life of the fish (bone) (Carleton and Martinez del Rio 2005; Carleton *et al*. 2008; Martinez del Rio *et al.* 2009). These data will be useful in studying both short and long term changes in prey assimilation.

GENERAL DISCUSSION

Recent trends in declining exploitable halibut biomass resulting from reduced size-at-age make biological and ecological studies essential to the sustainability of the halibut fisheries. These trends have led to continued reductions in harvest allocations for the commercial halibut fisheries, and more recently for the sport fisheries, leading to economic stressors for fishers and fishing communities. I explored time and cost efficient strategies for examining localized patterns in the declining size-at-age trends and methods for assessing proportional contribution of prey to halibut diet.

Size-at-age of Pacific halibut is currently assessed over broad regulatory areas using data from stock assessment cruises (IPHC 2012a). While smaller statistical areas are associated with samples, to our knowledge, these data are not included in current size-at-age analyses. Both sport and commercial fish are port sampled for length and age; these numbers are used to determine average sizes and ages of harvested fish and are not incorporated into size-at-age studies, presumably due to the bias associated with fish caught by sport fishermen and on long-line gear.

In order to fully understand the impacts of biological factors on size-at-age, I first compared the localized size-at-age in the sport fishery to the regional survey data. For females, size-at-age on in the Homer sport fishery was consistently larger than fish caught in the broader area, 3A, and fish caught in the same statistical area, 261. In most cases, males in our sample were also larger than those caught on IPHC stock assessment surveys. This is likely due to a combination of selective harvest of larger fish and differences in catch location. Regardless, with the knowledge

that our size-at-age was greater than those fish caught on stock assessment surveys, we were able to proceed with dietary sampling, keeping in mind that the results are more methodological than applicable to the region-wide biological trends. I port-sampled 740 fish over two summers (51 days) for size, age, sex, gape, stomach contents, and stable isotopes. I established cooperative partnerships with local government organizations and private businesses that made this sampling possible.

Researchers have sporadically studied halibut diet during stock assessment and other research cruises, but data are sparse and spatially scattered, likely due to the confines of labor intensive projects at-sea. Through port sampling, we were able to determine the frequency of occurrence of 46 prey species in the stomachs of 584 fish. There are many constraints to this method of dietary analysis. Nearly a quarter of all stomachs were empty, presumably due to regurgitation. Further, variable prey digestion rates (Hopkins and Larson 1990, Berens and Murie 2008) likely impacted our results. Finally, stomach contents only provide a "snapshot" of what the fish has recently consumed and may not be adequate for diet-growth studies. For these reasons, we explored the use of stable isotopes as an indicator of fish diet.

Stable isotopes have been used successfully to examine the diet of other fish species (Fry 1988, Benstead *et al.* 2006, Fernandez *et al.* 2011). Several tissue types can be used to assess dietary inputs on a variety of time scales, based on the tissue turnover rate of individual tissues (Sweeting *et al.* 2007, Caut *et al.* 2009, Hesslein *et al.* 1993). Here, we used muscle tissue due to the relatively long (several months) turnover rate and the efficiency of sampling and processing. Stable isotopes in halibut flesh indicated that there were significant differences in diet based on

all factors: sex, size, age, location and date of sample. Diet was constructed using a Bayesian implementation of a mixing model. Muscle tissues showed a wide range of potential proportional contributions of fishes, octopuses and crabs to halibut diet. In most cases, fishes appeared to contribute the most to diet, followed by octopuses, then crabs, with only minor contributions from amphipods. Results indicate that there may be some dietary differences between faster and slower growing fish. In particular, octopuses appear to contribute more for faster growing female fish. Future isotope studies should consider the use of $\delta^{34}S$ to improve the resolution of prey – consumer isospace.

Before stable isotopes are used for intensive diet sampling, a broader prey library must be established, preferably to include $\delta^{34}S$. It is likely that using prey values that are grouped differently to include a wider breadth of prey would improve the posterior distributions. Laboratory studies are also necessary to improve the state of knowledge of discrimination factors most appropriate for halibut studies. Stable isotopes are a promising tool for dietary studies and other studies related to halibut migrations and impacts of changing ocean conditions on halibut.

As changes in halibut size-at-age continue to impact harvest allocations, the mechanisms that cause reduced growth must be studied. In order for fish growth to slow, there must be changes in the quality or quantity of ingested energy, energy expenditure, or energy loss. The study of localized dietary inputs and their relations to growth will inform our understanding of ingested prey quantity. Expansion of studies to include energy and nutritional content of prey would further improve our knowledge of the quality of prey consumed. In addition to energy input, it will be important to study energy expenditure and loss. Several factors have been identified that

may be impacting energy expenditure in halibut including changes in water temperature and disease (Clark and Hare 1999, Kocan *et al.* 2010). Through port sampling, it is possible that such factors can be studied using additional isotopes ($\delta^{18}O$) and tissue samples. It is our hope that continued monitoring of halibut for ecological and biological data will improve managers' ability to make difficult decisions regarding halibut harvest limits and allocation.

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