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Assessing the Relationship between Diet and Size-At-Age in Pacific Halibut (*Hippoglossus stenolepis*) using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis

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Title: Assessing the Relationship between Diet and Size-At-Age in Pacific Halibut
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Head: Pacific Halibut Diet

Authors

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Abstract

The ecological mechanisms driving an observed decline in the mean size-at-age of adult Pacific halibut in the eastern North Pacific Ocean have yet to be defined. Here, we present the results of a study designed to investigate the relationship between one potential mechanism — diet — and size-at-age using carbon and nitrogen stable isotope analysis. Our results indicate significant differences in the skeletal muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between size-at-age categories for each sex, with larger-size-at-age fish consuming diets with higher $\delta^{15}\text{N}$ values, indicating higher trophic level feeding. Analysis of Bayesian standard ellipse areas showed that for females, intermediate size-at-age categories have the largest dietary range. For males, the largest dietary range was observed in the largest size-at-age category. Our results suggest a size-based stratification in dietary strategy for Pacific halibut with implications for observed declines in size-at-age.

Key Words

diet, growth, Pacific halibut, size-at-age, stable isotopes

Introduction

Since peaking in the 1970's, mean size-at-age of adult Pacific halibut (*Hippoglossus stenolepis*) in the eastern North Pacific Ocean has dropped by approximately 50% for both sexes (Stewart and Webster 2017, Clark and Hare 2006). While the impacts of this decline on stock biomass have been the subject of considerable focus (e.g. Stewart and Hicks 2017), the ecological mechanisms driving the decline have received less attention. Efforts to characterize these mechanisms have largely been hampered by a) the large spatial and temporal scales at which processes affecting the mean size-at-age of populations operate, and b) the inherently high individual variability in size-at-age observed in Pacific halibut (Forsberg 2017). Addressing the former of these challenges is largely a matter of data availability and is not possible at this time. However, developing an understanding of the ecological and physiological influences of growth, and consequent size-at-age, of individual Pacific halibut is possible using current analytical techniques and foundational to understanding the potential ecological drivers of population-scale changes in size-at-age. Here, we present the results of work designed to assess the relationship between one ecological variable and the size-at-age of individual Pacific halibut: diet.

Diet's role in influencing an individual's scope for somatic growth can be considered as a simple bioenergetic relationship, where scope for growth is a function of the energy remaining once metabolic and reproductive expenditures have been met minus energy lost to egestion and excretion of waste products. While this interpretation is a simplification and does not reflect the complex and dynamic interactions between environmental, ecological, and physiological processes (Jorgensen et al. 2015), it serves to demonstrate that increases in the quality or quantity of ingested feed can, minus the additional expenditures associated with catching, consuming, and processing (Mitra and Flynn 2005), result in additional energy available for growth (Zhou 2009). Consequently, we might expect individual Pacific halibut of a larger size at a given age to have consumed a greater quality or quantity of prey than smaller conspecifics. Because adult Pacific halibut are single prey-loaders, pursuing and consuming a single prey item at a time (rather than multiple prey items simultaneously), we

expect maximizations to net energy budgets to occur on an item-by-item basis (Lamb et al. 2017), thereby resulting in a stronger relationship between growth, and consequent size-at-age, and prey quality rather than prey quantity (*sensu lato* optimal foraging theory; MacArthur and Pianka 1966).

Previous examinations of Pacific halibut diets using stomach content analyses suggest a generalist dietary strategy, with fishes, crustaceans, mollusks, echinoderms, cephalopods, marine worms, kelp and fisheries offal as common prey sources (Moukhametov et al. 2008, St-Pierre and Trumble 2000, Best and St-Pierre 1986, Orlov and Moukhametov 2007, Roseneau and Byrd 2000). While stomach content analyses can provide definitive information on prey composition at a species-level, they have several important limitations. Stomach contents only provide information on what a fish has most recently consumed, and sometimes provide partial or no information due to regurgitation during capture. In addition, results from stomach content analyses are potentially biased due to rapid and variable digestion rates (Berens and Murie 2008). For example, crustacean exoskeletons typically remain in the stomach longer than soft tissues, such as muscle (Hopkins and Larson 1990). As a result, stomach content analyses merely describe prey items that have been ingested, rather than those that are digested, assimilated, and contribute to the energy budget.

Carbon (^{13}C) and nitrogen (^{15}N) stable isotope analysis offers a method to explore the diets of organisms that is not subject to the limitations of stomach content analysis. Because the carbon and nitrogen atoms in consumer tissues are entirely derived from dietary inputs (DeNiro and Epstein 1978, 1981), the proportions of carbon and nitrogen stable isotopes in a consumer tissue can be used to characterize the relative contributions of prey items or prey characteristics (e.g. trophic levels) assimilated over a unique temporal window based on the individual tissue's protein turnover rate (see references in Martinez del Rio et al. 2009). By focusing on prey that has been assimilated, rather than merely ingested, dietary analysis conducted using stable isotopes provides information on prey composition for a

longer time period than stomach content analysis and is not affected by regurgitation during the capture process or differential digestions rates.

We used ^{13}C and ^{15}N analysis to examine the dietary habits of Pacific halibut collected in the Lower Cook Inlet and nearby waters of southcentral Alaska. While previous studies (Hare and Kline 2003, Marsh et al. 2015) have used stable isotope analysis to explore the trophic position of Pacific halibut, ours is the first to use this technique to a) explore both the trophic level and the dietary range of Pacific halibut simultaneously, and b) investigate the relationship between diet and size-at-age in this species. Given Pacific halibut single prey-loading strategy, we expect larger size-at-age fish to exploit prey at a higher trophic level, but not necessarily a broader range of prey, than fish of a smaller size-at-age.

Methods

We sampled 588 individual Pacific halibut from the port of Homer in the lower Cook Inlet of Alaska, during May through September of 2012 and 2013. Homer supports the largest Pacific halibut sport fishery in the United States (Meyer and Powers 2013). Samples were collected from post-filleted carcasses donated by individual sport fisherman and sport charter fishing operations. For each halibut carcass, we determined the sex by visual inspection of the gonads and recorded fork length and jaw gape to the nearest cm. Blind-side sagittal otoliths were collected for age determination, and white muscle samples were collected from the lateral musculature for stable isotope analyses. Muscle were stored frozen at -20°C for analysis.

Otolith Aging

Otoliths were cleared for at least three weeks in a solution of 5.5g thymol, 20 ml 99.5% ethanol, 1.9L glycerin and 1.9L water (Forsberg 2001). Once clear, otoliths were broken radially through the nucleus. The dorsal half was baked at 500°F for 20 minutes in a conventional toaster oven. Otoliths were aged by counting dark concentric rings (winter growth) on the broken edge under 4X to 40X magnification using reflected light (Leica M60

stereomicroscope; Leica Microsystems Inc. Buffalo Grove, IL, USA). Quality control standards set by the International Pacific Halibut Commission (IPHC) call for an overall minimum age agreement of 33% and minimum age agreement within one year of 75% for break and bake aging. To determine aging precision, 79 otoliths were sent to the IPHC for age verification. Overall age agreement was 55.7%, and age agreement within one year was 83.5%.

Tissue Sample Preparation and Stable Isotope Analysis

Muscle tissue samples were thawed, oven-dried to a constant mass at 42°C, ground to a fine powder, of which approximately 0.5 to 1.0 mg was loaded into tin capsules for stable isotope analysis. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the tissue samples were determined using a Costech ECS 4010 elemental analyzer (Costech Analytical, Valencia, CA USA) coupled to a Thermo-Finnigan Delta V Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA USA) at the University of Alaska Anchorage Stable Isotope Facility (Anchorage, Alaska, USA) or using a Carlo Erba 1110 Elemental Analyzer (Carlo Erba Reagents, CE Instruments, ThermoQuest Italia S.p.A. Milan, Italy) coupled to a Thermo Delta Plus XP IRMS (Thermo Finnigan, Bremen, German) at the University of Wyoming's Stable Isotope Facility (Laramie, Wyoming, USA). Long-term analyses of quality control standards have yielded precisions of 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the University of Alaska Anchorage Stable Isotope Facility and 0.3‰ for $\delta^{13}\text{C}$ and 0.4‰ for $\delta^{15}\text{N}$ at University of Wyoming Stable Isotope Facility. Stable isotope data are presented in ‰ relative to Pee Dee Belemnite for ^{13}C and atmospheric nitrogen for ^{15}N .

Statistical Analyses

Relative size-at-age was characterized by grouping fish into quartiles based on proportional deviation from the median fork length for each age class (-1.00 to -0.51, -0.50 to -0.10, 0.00 to 0.50, and 0.51 to 1.00, hereafter referred to as size-at-age categories 1 to 4, respectively; Figure 1A). Age classes with fewer than five fish were excluded from our analyses, and males and females were grouped separately due to the strong sexual dimorphism in size

(Clark et al. 1999). Differences in gape between size-at-age categories were assessed for each sex using ANOVA tests followed by Tukey's HSD tests. MANOVA tests were used to assess differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between length quartiles.

Relative dietary ranges for each size-at-age category was assessed using Bayesian implementations of standard ellipse areas and centroid locations calculated using the Stable Isotope Bayesian Ellipses in R package (*SIBER*; Jackson et al. 2011) in the statistical software R (version. 3.4.1; R Core Team 2017). Standard ellipses were created using Bayesian estimates with the default *SIBER* settings, where Markov chain Monte Carlo length = 1,000,000, burn in = 1,000, thinning = 10, chains = 2, and 40% of the data is included in ellipse construction. Overlap among ellipses was assessed using Bayesian estimates of overlap expressed as proportions of the non-overlapping areas of the ellipses. Differences in the locations of ellipse centroids among size-at-age categories within each sex were assessed using pairwise comparisons of polar vectors.

Results

Of the 588 individual Pacific halibut sampled, 531 were in age classes that included ≥ 5 individuals and were, therefore, included in our analyses. Females accounted for 370 individuals, while males accounted for 161 individuals. Fork lengths and ages for females ranged from 47 to 200 cm (mean $\pm$ SD = 93 ± 24.63 ; range = 47 to 106, 52 to 127, 56 to 145, and 91 to 200 for size-at-age categories 1 to 4, respectively; Figure 1B) and 5 to 16 years (mean $\pm$ SD = 10 ± 2.59), respectively. For males, fork lengths and ages ranged from 52 to 95 cm (mean $\pm$ SD = 75 ± 7.43 ; range = 52 to 79, 59 to 82, 59 to 85, and 63 to 95 for size-at-age categories 1 to 4, respectively; Figure 1B), and 5 to 18 years (mean $\pm$ SD = 12 ± 3.10), respectively. $\delta^{13}\text{C}$ values ranged from -18.65 to -14.75‰ (mean $\pm$ SD = -16.76 ± 0.95 ‰) for females and -18.73 to -15.12‰ (mean $\pm$ SD = -17.36 ± 0.79 ‰) for males. $\delta^{15}\text{N}$ values ranged from 13.76 to 19.62 ‰ (mean $\pm$ SD = 15.87 ± 1.26 ‰) for females and 13.43 to 18.74 ‰ (mean $\pm$ SD = 14.95 ± 1.02 ‰) for males. Gape was recorded for 363 females and 159 males.

Gape size differed among the size-at-age categories for each sex ($F_{3,359} = 283.56$, $p < 0.01$ for females and $F_{3,155} = 6.37$, $p < 0.01$ for males), with category 4 fish exhibiting significantly larger gape size than category 1 fish (Figure 1C). This pattern was more pronounced in females than in males. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differed between size-at-age categories for each sex ($F_{6,732} = 23.98$, $p < 0.01$ for females and $F_{6,314} = 5.95$, $p < 0.01$ for males; Figure 2). For females, larger size-at-age categories had progressively higher nitrogen and carbon stable isotope values than smaller size-at-age categories (Figure 2). For males, this pattern was much less pronounced, with category 4 fish displaying $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that were significantly higher than smaller size-at-age categories, but similar to smaller category 2/3 females. All other males displayed little variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among size-at-age categories and were generally isotopically similar to the category 1 females (Figure 2).

All the Bayesian standard ellipse models converged and differences in the relationship between size-at-age and stable isotope values were also evident in the ellipse areas. For females, size-at-age categories 2 and 3 had larger ellipse areas than categories 1 and 4. For males, size-at-age category 4 had a larger ellipse area than all other categories (Table 1; Figure 3). Ellipse overlap differed among size categories. For females, overlap was highest between categories 2 and 3, with moderate overlap observed between categories 1 and 2, 2 and 4, and 3 and 4. Considerably less overlap was observed between categories 1 and 3 and 1 and 4. For males, overlap was highest among size-at-age categories 1, 2, and 3, with noticeably less overlap between these first three categories and category 4 (Table 2; Figure 3). Analysis of polar vectors between ellipse centroids among categories showed that within each sex, larger size-at-age fish tended to have higher carbon and nitrogen stable isotope values than smaller size-at-age fish. This difference was most pronounced when comparing the smallest (category 4) to the largest (category 1) fish in an age class (Table 3; Figure 3).

Discussion

Our results suggest differences in dietary habits among different size-at-age categories that, in turn, imply an influence of body size on the range of prey options exploited by, and possibly available to Pacific halibut. However, stable isotope analysis of muscle tissue does not establish a definitive link between diet and growth performance, only indicative. Previous investigations have interpreted $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as indicative of trophic position and carbon source, respectively (Jennings and van der Molen 2015; Post 2002). Isotopic variability among carbon sources can be the result of variations in dietary constituents or geographic space (Lorrain et al. 2011). Studies have reported rough latitudinal and inshore/offshore gradients in the $\delta^{13}\text{C}$ values of marine organic matter (Benner et al. 1997) and marine organisms (Radabaugh et al. 2013, Ruiz-Cooley and Gerrodette 2012, Lorrain et al. 2011, Cherel and Hobson 2007). Limitations in our understanding of both the spatial variability in ^{13}C in the Gulf of Alaska region of the North Pacific and the migration/movement of Pacific halibut in this region confine our interpretation of variability in the $\delta^{13}\text{C}$ values among the size-at-age categories of the Pacific halibut in our study. However, our results do suggest that for female Pacific halibut, larger size-at-age fish generally feed at a higher trophic position and exploit fewer prey types, or prey in fewer locations, than intermediate size-at-age fish. This is evident from the relatively smaller area and higher centroid $\delta^{15}\text{N}$ value of the Bayesian standard ellipses for category 4 females as compared to females in other size-at-age categories (Tables 1 and 3, Figure 3). Intermediate size-at-age females feed at a comparatively lower trophic position but exploit a larger dietary or geographical range as evidenced by the comparatively larger ellipse areas and lower centroid $\delta^{15}\text{N}$ values for categories 2 and 3 (Tables 1 and 3, Figure 3). In addition, the relatively high level of overlap between the category 2 and 3 Bayesian standard ellipses suggest strong similarity in the dietary or geographical ranges of these two size-at-age categories (Table 2, Figure 3). The relatively low centroid $\delta^{15}\text{N}$ value and small ellipse area of category 1 fish suggests that the smallest size-at-age females generally feed at the lowest relative trophic position, but, like the largest size-at-age females, also exploit a relatively narrow dietary or geographic range. Overlap of the Bayesian standard ellipses was highest between adjacent size-at-age categories (e.g. between categories 1 and 2, 2 and 3, 3 and 4,

etc.) with comparatively little overlap observed between categories 1 and 4, indicating divergence in dietary input with increasing differences in size-at-age (Table 2, Figure 3).

In contrast, male Pacific halibut display far less variability in both trophic position and range for all but the largest size-at-age fish. The Bayesian standard ellipse centroid $\delta^{15}\text{N}$ value and area were higher and larger, respectively, for size-at-age category 4 males; suggesting that these fish exploit a prey base that is generally superior in both trophic position and dietary or geographic diversity to smaller size-at-age males. This is also reflected in the relatively small amount of overlap between the ellipses for males in size-at-age category 4 and those in all other size-at-age categories (Table 2, Figure 3). Centroid $\delta^{15}\text{N}$ values, ellipse areas, and overlap indicate that males in size-at-age categories 1, 2, and 3 consume isotopically similar diets (Table 2, Figure 3). Comparison between the sexes suggests a similar prey range between males in size-at-age category 4 and intermediate size-at-age females. All other males consumed diets that were isotopically similar to females in category 1, the smallest size-at-age category.

By comparing the fork lengths of fish among different size-at-age categories (Figure 1B), we see that category 4 males are similar in absolute length to category 1 females. We also see similar patterns in gape size (Figure 1C). Consequently, we can conclude that a) males are similar to small females both in size and isotopically (Welch $t_{49.65} = 3.34$, $p < 0.05$ and Welch $t_{45.81} = 3.05$, $p < 0.05$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively), and b) dietary patterns, as indicated by stable isotope values, are related to absolute size regardless of sex.

The relationship between fork length and diet explains many of the patterns identified in our study. The small gape and short body size of the smallest fish physically limit their ability to consume larger or highly mobile prey, functionally limiting their dietary range to smaller, less mobile, and likely lower trophic level organisms. Successful halibut (i.e. those that can ingest more energy than is required to fulfill metabolic needs) grow, become less gape-limited, and experience increases in swimming speed and efficiency as a result of

increased body length (Takagi et al. 2010; Lighthill, 1969). Consequently, these larger fish may expend less energy to capture prey and can exploit a broader range of prey types or feeding locations; thereby increasing the potential to ingest more energy than is required for metabolic needs and resulting in further growth. Again, successful fish will grow and experience additional increases in swimming speed and efficiency allowing them to make use of high trophic levels and higher quality prey (Gerritsen 1984). With this increase in prey quality, fish again increase the potential to ingest more energy than is required for metabolic needs. While this results in further growth, we see a decline in the range of prey exploited, suggesting either moderate dietary specialization or limitation in geographic feeding range. While Pacific halibut are known to be highly mobile and migratory (Nielsen and Seitz 2017, Webster et al. 2013), there is currently little information on differences in short-term movement or migratory behavior among the adult size classes; thus, limiting our ability to draw inferences on differences in the potential relationship between size and the geographic footprint of feeding. In addition, although the specialization on higher trophic level prey observed in large size-at-age female Pacific halibut is in line with our expectation of a strong relationship between size-at-age and prey quality, our results cannot be explained entirely by the bioenergetic principles associated with optimal foraging theory (MacArthur and Pianka 1966). As single prey-loaders, we would expect a strict positive relationship between size and specialization under an optimal foraging paradigm. Instead, we observed increased dietary range with initial increases in size and specialization with subsequent increases. This contrast suggests that physical limitations (e.g. gape size) or ecological interactions (e.g. high levels of competition) may drive prey selection in smaller Pacific halibut, with energetic considerations becoming the primary driver once fish have reached a larger size threshold.

While this pattern of increasing trophic levels with size is not unusual in fish (Romanuk et al. 2011), the dietary specialization in the largest size-age female halibut group suggested by our results is contrary to the currently-held conception of Pacific halibut as dietary generalists (Moukhametov et al. 2008, St-Pierre and Trumble 2000, Best and St-Pierre 1986,

Orlov and Moukhametov 2007, Roseneau and Byrd 2000). This generalist classification does seem appropriate at the population level, our results question its applicability at an individual level. Further investigation of individual-level diet is possible using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and we recommend future studies examining the diets of Pacific halibut or, indeed, any marine fish species address the topic of potential differences in population-level vs. individual-level dietary strategy. In addition, our results demonstrate that spatial or temporal declines in high trophic-level prey, such as forage fishes, may result in declines in the ability of Pacific halibut to reach the largest size class; thereby resulting in regional or population-level decreases in size-at-age such as that observed in the eastern North Pacific Ocean (Clark and Hare 2006).

The relationship between diet and growth is one of several possible ecological mechanisms influencing the decline of size-at-age in Pacific halibut. Our results demonstrate a size-based stratification in dietary strategy with implications for the declines in size-at-age. As halibut move from a lower-quality diet with limited inputs to a more complex diet with considerably greater variety, and eventual reach a high-quality diet, they increase the potential for surplus energy available for growth. Our work demonstrates the importance of both dietary quantity (in the form of diversity of prey sources) and quality in fostering Pacific halibut growth. However, further investigation addressing the relationship between size and the geographic range of feeding is needed to refine our understanding of the range $\delta^{13}\text{C}$ values seen in our study.

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Table and Figure Legends

Table 1: Minimum, median, and maximum Bayesian standard ellipse area estimates for each size-at-age category by sex.

Table 2: Proportion overlap of ellipses among size-at-age categories for each sex.

Table 3: Polar vectors between ellipse centroids among size-at-age categories for each sex.

Figure 1: A) Relative size-at-age was characterized by grouping fish into quartiles based on proportional deviation from the median fork length for each age class (-1.00 to -0.51, -0.50 to -0.10, 0.00 to 0.50, and 0.51 to 1.00, referred to as size-at-age categories 1 to 4, respectively). Quartile boundaries are represented by dashed lines, and size-at-age category numbers are included in the top left of each quartile. Fork lengths (B) and gape sizes (C) for each size-at-age category for each sex.

Figure 2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\pm$ standard deviations) for size-at-age categories for females (top panel) and males (lower panel).

Figure 3: Bayesian standard ellipses and centroids for size-at-age categories by sex. Centroids are indicated by shapes and shape/line type indicate size-at-age category.

Table 1

Size-at-Age Category		Bayesian Standard Ellipse Area		
		Min	Median	Max
Females	1	1.44	2.08	3.16
	2	2.56	3.59	5.36
	3	2.37	3.73	4.61
	4	1.54	2.42	3.72
Males	1	0.79	1.35	2.76
	2	0.76	1.27	2.54
	3	1.26	1.89	3.22
	4	2.16	3.86	8.46

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Table 2

		Overlap as a Proportion of Non-Overlapping Area		
Size-at-Age Category		1	2	3
Females	1			
	2	0.50		
	3	0.39	0.70	
	4	0.28	0.46	0.62
Males	1			
	2	0.68		
	3	0.64	0.65	
	4	0.31	0.30	0.42

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Table 3

		Median Angle(°), Median Distance (‰)		
Size-at-Age Category		1	2	3
Females	1			
	2	0.97, 0.89		
	3	0.90, 1.50	0.79, 0.64	
	4	0.88, 2.15	0.82, 1.29	0.84, 0.67
Males	1			
	2	0.68, 0.28		
	3	0.81, 0.33	0.53, 0.31	
	4	1.03, 1.36	0.98, 1.33	1.07, 1.11

Figure 1

Figure 2

Figure 3