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Biochemical and Structural Factors Contributing to Seasonal Variation in the Texture of Farmed Atlantic Halibut (*Hippoglossus hippoglossus* L.) Flesh

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Factors contributing to the texture of fish flesh, including pH, water content, density of fast muscle fibers, and the concentration of collagen and hydroxylysyl pyridinoline (PYD) cross-links, were investigated post-rigor in commercially farmed Atlantic halibut (*Hippoglossus hippoglossus* L.). The fish was sampled every quarter for a 12 month period from May 2004 to May 2005. Hydroxyproline (HYP) as a measure of collagen and PYD were determined using a high-performance liquid chromatography (HPLC) method. An ANCOVA model with fork length and season as covariates were used to explore the seasonal effects on texture, pH, muscle fiber density, alkaline-insoluble collagen (a-i HYP), alkaline-soluble collagen (a-s HYP), and PYD cross-links. A multiple linear regression (MLR) showed that the most important factors contributing to texture were PYD > water (%) > a-i HYP > fiber density, while pH and a-s HYP did not show any correlation to texture. The contribution of fast muscle fiber density to texture was found to vary between sexes and with the season, contributing more in males and in the spring. The most important parameter affecting texture was PYD, explaining 64% ($p < 0.001$) of the total variation in a linear regression analysis. It is concluded that cross-linking processes are of great importance for the rigidity and strength of the collagen in Atlantic halibut flesh. Farmed halibut should be harvested in the fall or early winter when texture and nutrition are good to obtain optimal quality.

KEYWORDS: Farmed Atlantic halibut; seasonal effect; MLR analysis; collagen; cross-links; texture; pH; fiber density

1. INTRODUCTION

Texture is one of the most important sensory characteristics determining the eating quality of fish flesh. During the past decade, texture and the factors that are believed to influence texture have been intensively studied, including the effects of the season (*1*−*3*), stress (*4*), exercise (*5*), temperature and pH (*2*, *6*), chemical composition (*7*, *8*), muscle cellularity (*9*−*11*), and collagen content (*12*−*14*). The texture of fish flesh also varies with body size (*8*) and between species (*14*). This underlies the importance of research on each of the major commercial species, particularly farmed fish, where there is a measure of control over the rearing and slaughter conditions. Atlantic halibut (*Hippoglossus hippoglossus* L.) is one of the marine species that is being considered for large-scale farming in Norway, but little work has been performed on understanding the textural characteristics of its flesh (*15*, *16*).

The extracellular matrix (EMC) of fish has a complex organization and compromises several molecular species, including collagen, noncollagenous glycoproteins, and proteoglycans, often referred to as ground substances. The bulk of the collagen in fish is located in the myocommata separating the muscle segments, the myotomes. Apart from the myocommata, collagenous sheets surround individual muscle fibers (endomysium) and bundles of muscle fibers (perimysium) (*17*). The collagen superfamily comprises 85% of the relative area [calculated using transmission electron microscopy (TEM)] of the EMC (*18*). There are thus far 27 known collagen types with a tissue-specific expression pattern (*19*). Fish skeletal muscle has only ∼3% of the collagen content of mammalian muscle (*20*). Collagen type I and V have been identified, respectively, as the major and minor constituents of fast muscle, which comprises the major portion of fish fillets (*20*). The relative concentration of type V collagen is higher in the endomysium fraction than in the myocommata fraction compared to type I collagen (*21*). In addition to collagen type I and V, collagen types III, IV, and VI have also been characterized in fish (*22*), and the amount of collagen in the EMC is known to vary considerably between species (*12*, *14*, *23*). The myocommata has been reported to increase in thickness with age (*24*), but relatively little is known about changes in its molecular

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Table 1. Biological Data (Fork Length, Body Mass, and Sexual Distribution), Analyzed Variables, and Their Changes According to the Season[a]

	24.05.04	20.08.04	26.11.04	18.02.05	05.05.05
sex (number of fish)	13♀/7♂	9♀/11♂	11♀/9♂	10♀/10♂	13♀/7♂
body mass (g)	1255 ± 275	1942 ± 314	2208 ± 344	2036 ± 438	2079 ± 538
fork length (cm)	47.1 ± 3.0	53.6 ± 2.9	55.0 ± 2.4	55.6 ± 3.4	57.0 ± 2.9
shear work (mJ)	82.7 ± 29.2	66.7 ± 8.9	76.0 ± 27.1	98.1 ± 27.4	134.6 ± 28.4
pH	6.48 ± 0.1	6.45 ± 0.1	6.33 ± 0.1	6.34 ± 0.1	6.47 ± 0.1
FD (fibers/mm^2)	293 ± 40	252 ± 46	229 ± 41	233 ± 34	277 ± 57
a-s HYP (μmol g^{-1} dry mass)	12.6 ± 2.6	12.5 ± 2.9	7.0 ± 3.8	6.3 ± 2.3	12.3 ± 4.5
	(∼51%)	(∼53%)	(∼39%)	(∼32%)	(∼45%)
a-i HYP (μmol g^{-1} dry mass)	12.2 ± 4.9	11.2 ± 3.1	10.9 ± 3.8	13.2 ± 4.5	14.9 ± 6.9
	(∼49%)	(∼47%)	(∼61%)	(∼68%)	(∼55%)
total collagen	24.7 ± 6.0	23.7 ± 5.1	17.9 ± 6.3	19.5 ± 3.6	27.2 ± 9.4
PYD cross-links (pmol g^{-1} dry mass)	1029 ± 308	849 ± 138	934 ± 167	1071 ± 248	1458 ± 415
ratio PYD/A-i HYP (∼)	1:12 000	1:13 000	1:12 000	1:12 000	1:10 000

[a] Shear work (mJ), post-rigor pH, muscle fiber density (FD), alkaline-soluble (a-s) and alkaline-insoluble (a-i) HYP, total HYP, and hydroxylysyl pyridinoline (PYD) cross-links. Numbers in parentheses are the percentage of total HYP. Values represent the mean ± standard deviation (SD).

composition [see the reviews of Bailey et al. (*25*) and Bailey (*26*)]. The most important and striking alteration in the ECM with age is probably the formation of matured intermolecular cross-links between collagen fibers (*26*). Two main mature cross-linking processes have been described: one enzymatic step involving lysyl oxidase and one nonenzymatic step, a process called glycation (involving a reaction with glucose) (*25*). These mature cross-links contribute toward the maintenance of the physical structure and rigidity of the collagen matrix, and recently, the mature cross-link species, hydroxylysyl pyridinoline (PYD), was shown to be positively correlated to texture in raw and smoked Atlantic salmon (*Salmo salar* L.) flesh (*12*, *13*).

The overall aim of the present study was to investigate the relative contributions of post-rigor pH, muscle cellularity, and the concentrations of collagen and pyridinoline cross-links to the texture of flesh in farmed Atlantic halibut in relation to the season of slaughter. Because Atlantic halibut show sexual dimorphism in growth and muscle fiber recruitment patterns (*27*), the influence of sex on the texture and chemical composition of the flesh were also investigated.

2. MATERIALS AND METHODS

2.1. Fish Husbandry. The farmed Atlantic halibut (*H. hippoglossus* L.) used in this study were provided by the commercial production of Aga Marin on Dønna (Helgeland, Norway). The fish originated from Brandal Havbruk AS (Brandal, Norway) (2001) and were on-grown for 1 year (2002) before being sold to Aga Marine AS. The fish was reared under ambient conditions in 15 × 15 × 8 m netpens and given formulated feed, Biomarine kveite (50% crude protein, 22% crude fat, 6% nitrogen-free extract, 0.3% crude fiber, and 8.6% ash; data provided by BioMar AS, Trondheim, Norway), distributed with an automated feeding system. During the trial, 20 fish were sampled once every 3rd month from May 2004 to May 2005 ($n = 5$, see **Table 1**). A total of 10 fish were randomly selected from each of the two netpens in the afternoon, killed with a sharp blow to the head, and stored on ice until the next day. A total of 100 fish were sampled. Fish were shipped to Bodø on the public speed boat, arriving at Bodø University College, approximately 20 h post-mortem. Biological data and samples for morphometric studies were taken [see Hagen et al. (*27*)], and the fish were stored on ice for a total of 4 days in a cold chamber (2 °C) in plastic bags until further processing post-rigor. Fast muscle fiber density was calculated as followed: $FN_{measured}/area_{cumulative}$, where $FN_{measured}$ is the number of fast muscle fibers and $area_{cumulative}$ is the cumulative muscle cross-sectional area in mm^2 measured.

2.2. Instrumental Texture Measurement. Texture (shear work) measurements were made at 0.55 fork length (see **Figure 1**). The texture of the flesh was determined in duplicate using a TA-XT2 texture analyzer with Texture Expert Exceed 2.52 software from Stable Micro Systems (Surrey, U.K.) using a shear test [see Johnston et al. (*28*)]

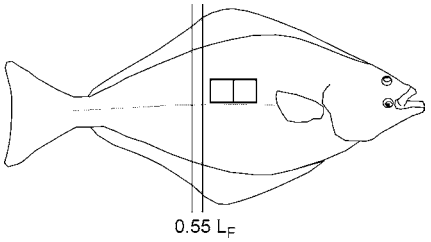

Figure 1. Sample sites for instrumental texture measurement (quadrangles) and tissue samples for the collagen and cross-link assays. Tissue samples for the collagen and cross-link were taken from the minced fillet anterior to the 0.55 L_F cut on both the dorsal and ventral sides of the body.

Muscle blocks were cut from the deeper layer of dorsal fast myotomal muscle using a standardized frame (2.5 × 2.5 × 1 cm) (**Figure 1**). The fish were filleted in groups of five and kept on ice prior to texture measurements to ensure fillets were not exposed to temperature fluctuations. The probe used in the shear test was a 60° knife-edge blade (not sharpened). A 25 kg load cell was used, and the test speed was set to 1 mm s^{-1}. The texture profile was analyzed using the Texture Expert Exceed 2.52 software, to determine maximum shear force, described as the highest peak value measured in Newtons (N), while the area under the curve during shearing until fracture corresponds to the work performed in millijoules (mJ) (*29*).

2.3. Sample Preparation. The location of samples taken for biochemical analyses is shown in **Figure 1**. Red muscle close to the horizontal septum was discarded, and the fast (white) muscle was minced using a food processor for 1 min at full speed (10 000 rpm). The pH of the mince was measured using a PHM92 pH-meter (Radiometer Analytical, Copenhagen, Denmark). A total of 1 g of the mince was accurately measured into four 50 mL centrifuge tubes, which were stored in a −80 °C freezer until the determination of hydroxyproline (HYP) and PYD. Muscle water content were analyzed in duplicates of 5 g of mince, estimated as the weight loss at 104 °C after 16 h.

2.4. Collagen and Cross-link Assay. The assay used for collagen and cross-link analyses is a previously described method, using high-performance liquid chromatography (HPLC) (*13*). Briefly, 9 mL of cold distilled water (4 °C) was added to each of the 50 mL tubes containing exactly 1 g of muscle and homogenized (25 000 rpm) for 1 min using a Polytron (modified PT 1200CL, Kinematica AG, Littau, Switzerland). Then, 10 mL of cold 0.2 M NaOH was added to each, and the tubes were placed on a rotator (Stuart rotator SB3, Bibby Sterilin LTD, Straffordshire, U.K.) in a cold room (2 °C) for 4 h. Post-rotation, the tubes were centrifuged at 10000*g* for 30 min at 4 °C, and both the supernatant [alkaline-soluble (a-s) HYP] and pellet [alkaline-insoluble (a-i) HYP] were hydrolyzed in 6 M HCl at 110 °C for 20 h. After hydrolysis, 10 μL a-s HYP and 2 μL a-i HYP were then removed and

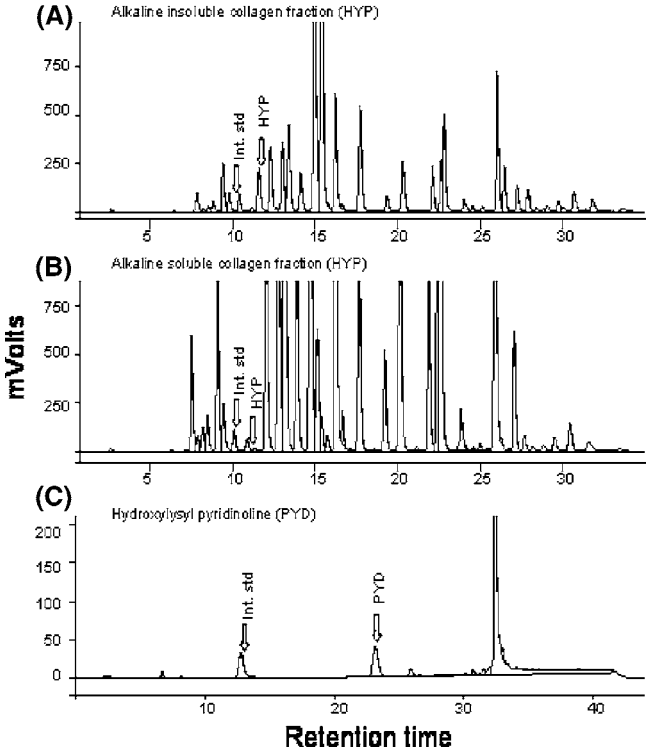

Figure 2. Chromatogram showing the elution profiles and retention times of hydroxyproline in the (A) alkaline-insoluble and (B) alkaline-soluble tissue fractions (homoargenine was used as an internal standard) and (C) the PYD cross-link fraction (pyridoxine was used as an internal standard).

resuspended in distilled water for HYP analysis. The remaining hydrolyzed a-i collagen fraction was used for PYD cross-link analysis and concentrated together with the HYP samples using a vacuum rotary evaporator (Jouan RC1022, Nantes, France). For PYD extraction, a cellulose column (Varian Ltd., Oxford, U.K.) was used (*30*, *31*). After extraction, samples were resuspended in injection sample buffer (5% CH_3CN and 1% HFBA) containing 250 nM pyridoxine (internal standard, Fluka) and filtered [0.2 μm polyvinylidene difluoride (PVDF) membrane] before being transferred to the HPLC in glass vials. The HPLC system, column, and eluents used are the same as described by Li et al. (*13*), except that an upgraded 240 Inert SDM solvent-delivery module was used. Excitation (λ_{ex}) and emission (λ_{em}) wavelengths for PYD analysis were set to 295 and 400 nm, respectively. Results were calculated on the basis of a standard curve made of purified PYD (kindly gifted by Simon Robbins, Rowlett Institute, Aberdeen, U.K.) using the Star system control version 6.30 software (ProStar, Varian Analytical Instrument, Walnut Creek, CA).

The samples for the HYP analysis were resuspended in 0.1 M borate buffer (pH 11.4) containing homargenine (10 μM) as an internal standard and derivatized using fluorenylmethoxycarbonyl (FMOC) (*32*). Before the HYP samples was loaded onto the HPLC, they were resuspended in injection buffer consisting of 25% (v/v) CH_3CN in 0.25 M boric acid (pH 5.5). Quantification of the homoarginine and hydroxyproline peaks was based on the standard curve made from collagen hydrolysate (Sigma). Excitation (λ_{ex}) and emission (λ_{em}) wavelengths were set to 254 and 630 nm, respectively. The chromatograms for a-i and a-s HYP and PYD are shown in **Figure 2**.

2.5. Statistics. The statistical tests were preformed using Minitab (version 13.20, Minitab, Inc., State College, PA) and SigmaPlot (version 8.0, SPSS, Inc., Chicago, IL). The size distribution of the fish was analyzed using a general linear method (GLM). An ANCOVA model (independent variable = season + sex + season × sex) was used to analyze the seasonal effects on the variables. The fork length and season were used as covariates. PYD and texture data (pooled data) were subjected to a square rot transformation, while a-i HYP were subjected to a log transformation (left skew of the distribution) to comply with the assumptions for the analysis. Scatterplot matrices of all independent variables were used to study the relationships between them. All independent variables and their relative importance to texture (dependent variable) were further explored using a multiple linear regression (MLR). A connection between PYD and water content and a-i HYP was observed from the scatterplot matrices. To overcome the overlapping effect of one variable on another, two MLRs were preformed: one with PYD included, excluding a-i HYP and water, and one GLM without PYD. A linear regression analysis was used to show the most important relationships graphically.

3. RESULTS

3.1. Seasonal Variation in Texture and pH. Fillet texture was not significantly different between male and female fish ($p = 0.462$, ANCOVA), and data were therefore combined (**Table 1**). There was a significant seasonal effect on texture during the trial ($p < 0.001$, ANCOVA). Texture was similar in May and August 2004 but increased by 2-fold from 66.7 to 134.7 mJ between August 2004 and May 2005 (**Table 1**). Fast muscle pH did not show any significant seasonal variation or variation between sex ($p > 0.1$, ANCOVA).

3.2. Effect of the Season on Muscle Collagen and Pyridinoline Cross-link Concentrations. No differences in the a-s HYP or a-i HYP concentrations were observed between male and female halibut ($p > 0.2$, ANCOVA), and data were therefore combined for further analysis of seasonal effects. There was a significant seasonal effect on the concentration of a-s HYP ($p < 0.001$, ANCOVA) (**Table 1**). The a-s HYP was similar between May and August 2004 (**Table 1**) but decreased 2-fold by mid-winter the following year. In May 2005, the a-s HYP concentration was not different from that of May 2004 (**Table 1**). The seasonal effect on the a-i HYP fraction showed a similar trend to that of a-s HYP but not as obvious, decreasing during the fall and increasing during the winter, having the highest value in May 2005 ($p < 0.01$, ANCOVA) (**Table 1**).

The concentration of PYD showed a highly significant seasonal effect ($p < 0.001$, ANCOVA). The concentration of PYD in May and August 2004 was similar but increased by ~70% in May 2005 (**Table 1**). In the same period, a drop in the total protein content was seen, being most obvious in males (~20%) [$p < 0.005$, see Hagen et al. (*27*)]. Thus, the protein fraction of the fast muscle was decreasing and becoming enriched for PYD during the winter ($r^2 = 0.64$, $p < 0.001$, not shown). At the same time, an increase in water content was seen, with a difference between sexes during the late winter [see Hagen at al. (*27*)], because of a depletion in males. Water content also showed a highly significant correlation to PYD ($r^2 = 0.61$, $p < 0.001$, not shown).

3.3. Impact of pH, Fiber Density, Water Content, Collagen, and Pyridinoline Cross-link on Texture. To identify which independent variables affected texture, pH, fiber density, a-i HYP, a-s HYP, water, and PYD were further investigated using MLR, with backward exclusion of independent variables. Because PYD was found to correlate with water and a-i HYP, two MLRs with PYD, excluding a-i HYP and water, and one only without PYD were preformed. The outcome of the MLR showed that the independent variables most important to texture were PYD > water (%) > a-i HYP > fiber density, while pH and a-s HYP did not show any correlation to texture. A linear regression analysis was preformed to show the relationships between the dependent and the most important independent variables. The water content of the flesh correlated with the texture of females (**Figure 3A**) and males (**Figure 3B**), explaining 53 and 60% of the total variation, respectively.

Muscle fiber density was found to be significantly different between males and females ($p < 0.01$, $n = 98$, ANCOVA, not

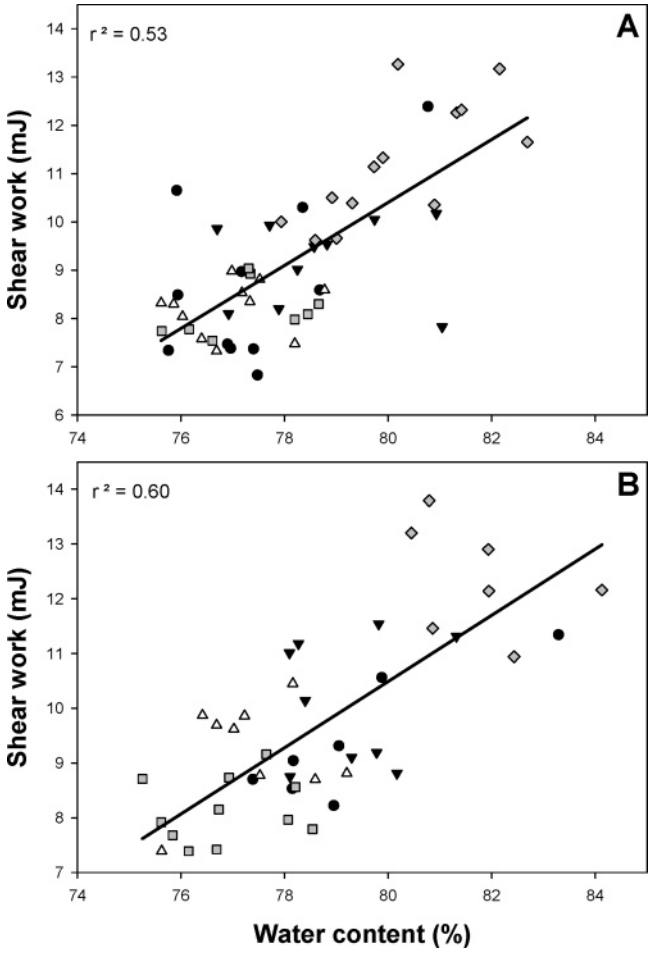

Figure 3. (A) Linear regression analysis of water (%) and texture (square root transformation) in females ($n = 44$, $r^2 = 0.53$, and $p < 0.001$). A first-order linear regression was fitted to the data; shear work = 0.653 (water) − 41.804. (B) Regression analysis of water (%) and texture in males ($n = 54$, $r^2 = 0.60$, and $p < 0.001$). A first-order linear regression was fitted to the data; shear work = 0.605 (water) − 37.924. Symbols indicate the different samples: 24.05.04 (●), 20.08.04 (■), 26.11.04 (△), 18.02.05 (▼), and 05.05.05 (◆).

shown). On a group level, the impact of the fiber density on texture changed during the season, ranging from being not significant in August and November 2004 to being significant in May 2005, explaining 31% of the total variation ($r^2 = 0.31$, $p < 0.001$, $n = 20$) (not shown). When males and females were plotted separately, males displayed the strongest correlation ($r^2 = 0.19$, $p < 0.01$, $n = 44$), explaining ~19% of the variation (**Figure 4**). The total HYP fraction displayed a weak correlation to fiber density ($r^2 = 0.14$, $p < 0.01$, pooled data, $n = 98$, not shown).

The a-i HYP fraction made a similar contribution to texture in both male and female fish, and the pooled data explained ~24% of the total variation (**Figure 5A**). On the other hand, a-s HYP did not correlate with texture and the total HYP fraction showed a weaker significant correlation ($r^2 = 0.15$, $p < 0.001$, $n = 98$, not shown) than the a-i HYP fraction. The single parameter showing the strongest correlation to texture was the concentration of PYD, explaining as much as 64% of the total variation (**Figure 5B**). All equations for the linear relationships are given in the respective figure captions. The PYD concentration increased with the age of the fish, and this was reflected as an increase in fillet firmness between May 2004 and 2005 (**Table 1**).

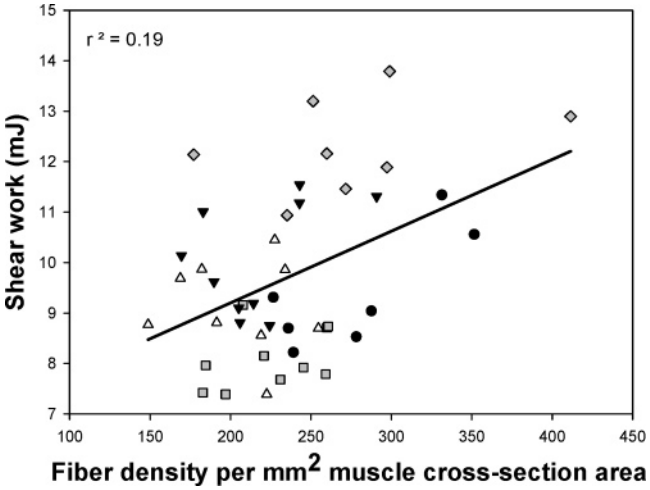

Figure 4. Regression analysis of the fiber density (FD) and texture (square root transformation) in males (pooled data, $n = 44$, $r^2 = 0.19$, and $p < 0.01$). A first-order linear regression was fitted to the data; shear work = 0.014 (FD) + 6.356. Symbols indicate the different samples: 24.05.04 (●), 20.08.04 (■), 26.11.04 (△), 18.02.05 (▼), and 05.05.05 (◆).

4. DISCUSSION

This study has shown that mature PYD cross-links make a major significant contribution to fillet firmness in the Atlantic halibut, explaining 64% of the total variation (**Figure 5B**). There are very few studies on collagen cross-links in fish muscle. In a recent study, the PYD concentration was shown to be positively correlated with firmness in fresh and smoked salmon, explaining 25 and 16% of the variation, respectively (*13*). It is likely that the a-s and a-i fractions contain native and cross-linked collagen, respectively, because almost 100% of PYD cross-links were recovered in the a-i fraction (*13*). Johnston et al. (*12*) reported that a wild salmon population had significantly firmer flesh than a farmed population and yet had similar concentrations of PYD cross-links. This is probably because other mature and immature cross-link species contribute to texture together with the protein compartment (*10*, *12*). Other immature cross-links include dihydroxy-, hydroxy-, and lysinonorleusine, and mature cross-links include deoxypyridinoline and pentosidine (*33*). It is assumed that one collagen molecule consists of ~200 HYP residues, and one PYD is capable of connecting three collagen molecules (*13*). On the basis of these assumptions, ~6 and ~3% of the a-i and total collagen were cross-linked in the final sample, respectively, compared to just 1% in Atlantic salmon (*13*). Thus, in comparison to salmon (*12*, *13*), halibut have a more densely cross-linked collagen and firmer flesh (present study). No correlation between texture and the a-s HYP fraction, which is thought to represent native non-cross-linked collagen, was found for either halibut (present study) or salmon (*13*).

Farmed Atlantic halibut largely cease feeding in the winter because of the low temperature (<6 °C) and short days, resulting in a mobilization of fast muscle proteins and an increase in water content (*27*). This seasonal depletion is particularly severe for males of the body size used in the present study because of precocious sexual maturation, which results in additional muscle protein mobilization to build up the gonads (*27*). In the present study, a significant seasonal effect in both soluble collagen ($p < 0.001$) and insoluble collagen ($p < 0.01$) was found but not as obvious in insoluble as in the soluble collagen fraction. It is likely that the cross-linked collagen fraction is more resistant

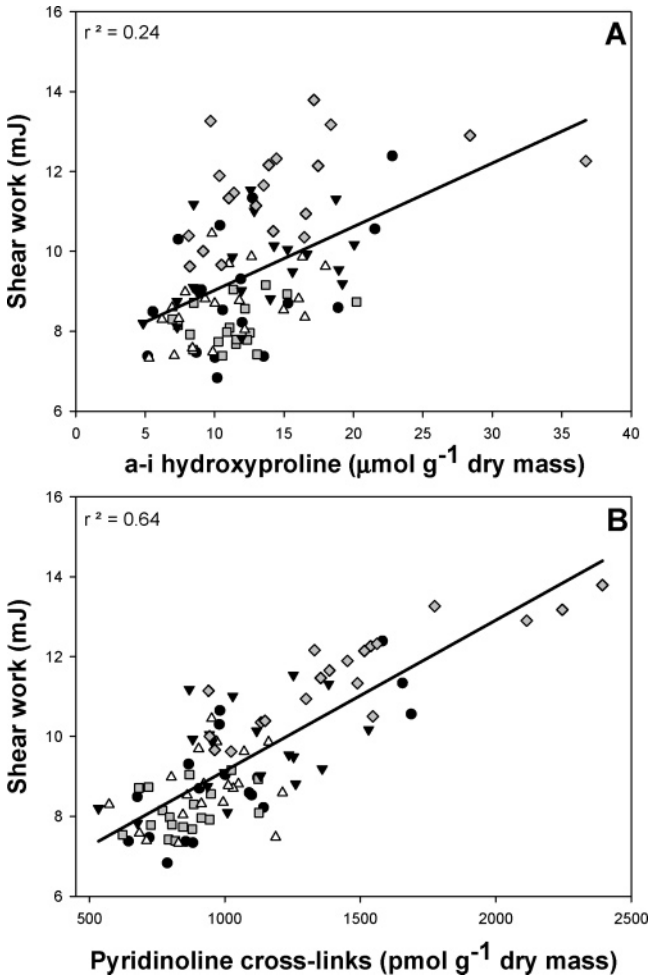

Figure 5. Relationship between (A) alkaline-insoluble collagen ($n = 98$, $r^2 = 0.24$, and $p < 0.001$) and (B) PYD ($n = 98$, $r^2 = 0.64$, and $p < 0.001$) and the fillet texture (square root transformation) for Atlantic halibut. A first-order linear regression was fitted to the data; (A) shear work = 0.159 (a-i HYP) + 7.432, and (B) shear work = 0.04 (PYD) + 5.361. Symbols indicate the different samples: 24.05.04 (●), 20.08.04 (■), 26.11.04 (△), 18.02.05 (▼), and 05.05.05 (◆).

to proteolytic breakdown by collagenases (*34*). Red seabream showed no seasonal change in the collagen concentration but an increase with maturation and spawning (*35*). This is in sharp contrast with the decreased collagen concentration observed during maturation and spawning in pacific herring (*36*), Aya (*37*), and halibut (present study). In the present study, all male halibut matured, while females did not (*27*). During the maturation period, females outgrew males, resulting in them being significantly larger in May 2005 (*27*). Because both sexes showed the same changes in the insoluble collagen concentration, it is likely that seasonal factors rather than maturation are responsible. PYD is known to increase with age in both mammals (*25*, *26*) and fish (*38*). The reason why the contribution of water content to texture showed a small difference between sexes is that males displayed a more pronounced increase in water content than females during the winter (*27*). PYD cross-links are more concentrated in the fish muscle with increased water content ($r^2 = 0.61$, pooled data). However, the difference in the PYD to a-i ratio was not significant.

A weak significant correlation was found between total collagen and muscle fiber density, as reported in seabream (*11*). This probably reflects the higher surface to volume ratio of endomysium as muscle fiber density increases. However, the relative contribution of the endomysium collagen fraction is probably relatively small because most of the collagen in fish muscle is located in or associated with the myosepta.

The contribution of other variables to the texture of halibut flesh was explored using MLR. Muscle fiber structure and texture are known to correlate in both raw (*10*, *11*) and cooked fish (*9*) using taste panels and instrumental methods. In the present study, a linear regression analysis revealed that fast muscle fiber density in males (**Figure 4**, $r^2 = 0.19$, $n = 44$) correlated better with texture than that in females, perhaps reflecting the sexual dimorphism of muscle fiber recruitment patterns in this species (*27*). During the season and between sampling points, the contribution of fiber density to texture varied from not being significant (August and November 2004) to having a larger effect in May 2005 ($r^2 = 0.31$, $n = 20$), implying that muscle fiber density is just one of the factors affecting texture.

pH has been reported to influence the texture of fish muscle, particularly in relation to the phenomena referred to as gaping (splits and tears in the connective tissue) (*18*), although its impact on technological characteristics of the flesh have been debated (*1*, *2*). On the basis of the present findings, it is concluded that post-rigor pH is not a good predictor of texture in raw Atlantic halibut flesh.

In conclusion, collagen cross-linking constitutes a major factor and muscle fiber density constitutes a minor factor explaining fillet firmness in Atlantic halibut as measured with an instrumental texture analyzer. Significant seasonal effects were observed in both collagen cross-links and muscle fiber density, which had an impact on texture, and their relative importance varied during the year. During the winter, when growth ceased, the a-i collagen fraction was enriched for PYD cross-links, leading to a firmer texture. In practical terms, harvesting during the spring would result in fish with the firmest texture. However, the fish would loose biomass because of maturation and muscle depletion during the winter months (*27*), and therefore, we recommend harvesting in the fall or early winter when the nutritional state (*27*) and texture are good.

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