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Activity of Aspargate (Cathepsin D), Cysteine Proteases (Cathepsins B, B + L, and H), and Matrix Metallopeptidase (Collagenase) and Their Influence on Protein and Water-Holding Capacity of Muscle in Commercially Farmed Atlantic Halibut (*Hippoglossus hippoglossus* L.)

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Atlantic halibut (*Hippoglossus hippoglossus* L.) were commercially farmed in Helgeland, Norway (May 2004−May 2005). The average weight (Mb) of fish increased over the 12 month production cycle by ∼73% for females and ∼50% for males, although during the winter months (November−early May) Mb was unchanged in females and declined by 18% in males because of sexual maturation and sperm release. Periods of zero or negative growth were associated with up to 5.7% (females) and 17.9% (males) decline in fast muscle protein content. The activities of cathepsins B, B + L, H, and D showed a reciprocal relationship and were highly correlated with the changes in protein content. Water-holding capacity was measured as the liquid loss increased from 3−5% in November to 11−13% in May. Two general additive models (GAMs) showed that cathepsin B + L, cathepsin D, and collagenase explained 73.1% of the total variance in protein content, while cathepsin H was the largest contributor to liquid loss, explaining ∼48.8% of the total variance. The results indicate that to obtain the best flesh quality Atlantic halibut should be harvested in the fall or early winter when the liquid loss and cathepsin activities are low and less likely to cause problems during secondary processing and storage.

KEYWORDS: Farmed Atlantic halibut; seasonal effect; water-holding capacity; cathepsin; collagenase

1. INTRODUCTION

Fish muscle contains 60−80% water depending upon species and various biological and environmental factors (*1*). Water-holding capacity (WHC) is therefore not surprisingly an important quality parameter for both industry and consumers (*2, 3*). Muscle proteins have a central role in the binding of water, originating in the unequal distribution of positive and negative charges of water molecules (*4*). The interactions between water and proteins can be divided into three categories based on binding: (A) primary hydration shell (bound water), (B) secondary hydration shell (loosely bound water), and (C) unperturbed water (free water) (*4*). Variation in the WHC of the flesh is due to how well the physically bound water is locked up in the muscle structure (*4*). When commercially produced fish are harvested, WHC should be taken into consideration because the drip loss can be considerable. In aquaculture, problems of drip loss can potentially be minimized by manipulating conditions (e.g., feed, light regimes, and potentially temperature) as well as harvest time to obtain fish of the best quality. Several small-scale experiments has been performed to investigate the WHC of current Norwegian aquaculture species, including salmon (*Salmo salar* L.) (*5, 6*), cod (*Gadus morhua* L.) (*5, 7*), and Atlantic halibut (*Hippoglossus hippoglossus* L.) (*8–10*). However, the optimal timing of harvesting with respect to WHC has not yet been addressed in commercially produced halibut. Several parameters have been associated with poor WHC, such as ionic strength, pH, temperature (*6, 9*), detachment of sarcolemma, gaps in the extracellular matrix, widening of the intermyofibrillar space, and transversal shrinkage of the muscle fibers (*10*). The presence of certain bacteria during cold storage is also known to affect the WHC of the flesh (*7, 10*). The majority of the water in the flesh is bound to the myofibrils, and the WHC of the muscle is linked to the spacing of the myofibrils (*11*). Any proteases capable of degrading muscle proteins could potentially affect the WHC of the muscle.

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Cathepsin activity has been studied in fish in relation to *post mortem* autolysis (*12–14*), the effect of ice storage (*15*), smoking temperature (*16*), and season and fishing ground on the byproduct (*17*). In addition, the activity of cathepsins has been explored in relation to the degradation of proteins and softening of muscle tissue, which has showed to lead to reduced quality during starvation and maturation (*18, 19*). The cathepsins (B, B + L, and H) belong to the cysteine peptidase family, a group of enzymes that are associated with the lysosomes in many tissues, including muscle. Cathepsin D on the other hand belongs to the aspartic peptidase family [see review of Sentandreu et al. (*20*)]. Cathepsins show a range of substrate specificities. For example, myosin heavy chain (*21*), troponin I, troponin T, and tropomyosin (*22*) are all degraded by cathepsin B. In addition, cathepsin B (B1) is also thought to degrade native collagen (*23*). Cathepsin L is capable of degrading most myofibrillar structural proteins, such as myosin, actin, and troponin I and T but not troponin C and tropomyosin (*12, 24*), while cathepsin H is known to degrade troponin T (*25*). A light microscopic study provided evidence that cathepsin L was involved in the degradation of the M protein in the M line and structural components of the Z line (*26*). The regulation of collagenases are complex, but they are synthesized and secreted in a number of cells, including muscle fibers and fibroblasts (*27, 28*). The matrix metalloprotease peptidase family, including collagenase has specific functions with particular specificity toward collagen substrates (*20*).

The main objective of the present study was to investigate the activity of cathepsins B, B + L, D, and H and collagenase over an annual production cycle in commercially produced Atlantic halibut and determine their impact on protein content and liquid loss. On the basis of the results, an optimal timing of harvesting to minimize liquid loss and maximize flesh quality is suggested.

2. MATERIALS AND METHODS

2.1. Fish Farming. Farmed Atlantic halibut (*Hippoglossus hippoglossus* L.) were supplied from Aga Marin's commercial production (Dønna, Helgeland, Norway). A total of 20 fish were randomly sampled once every third month ($n = 100$ fish) from May 2004 to May 2005. The fish were farmed under ambient conditions in 15 × 15 × 8 m netpens containing a shelf system (7 shelves of 25 m^2 each), and feed (BioMarine kveite) was distributed with an automated feeding system. The fish were killed by a sharp blow to the head in the afternoon (3 pm), stored in polystyrene boxes on ice overnight, and transported with the local speedboat to Bodø University College the next morning (~20 h *post mortem*). See Hagen et al. (*29, 30*) for more information about farming conditions.

2.2. Sample Preparation. Upon arrival, all biological data were recorded and the fish were stored in plastic bags on ice in a cold room (2 °C) until 4 days *post mortem* (*post rigor*). The fish were then rinsed and filleted (dorsal and ventral side) anterior to 0.55 L_F and minced for 3 × 5 s using a food processor, and a fraction was removed and used for WHC analysis (see below). The remaining mince was homogenized for an additional 1 min, and 1 g was accurately measured in 15 tubes per fish and stored in a −40 °C freezer until enzyme analysis was preformed.

2.3. WHC. The WHC was measured according to Ofstad et al. (*5*). Briefly, the mince was kept on ice at all times, and 15 g was accurately measured in duplicate into a cup with a fine netting bottom. The cup was placed on top of a collection vessel and centrifuged at 210 g for 15 min at 10 °C. WHC was measured as the liquid loss and expressed as a percentage of weight released (*7*). Because of the low fat content of the samples [see Hagen et al. (*29*)], the potential "fat fraction" of the liquid post-centrifugation was ignored.

2.4. Enzyme Kinetics. *2.4.1. Cathepsins B, B + L, and H Activity Assay.* A total of 1 g of muscle measured into tubes was homogenized in 5 mL of cold extraction buffer [50 mM sodium acetate, 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.2% (w/v) 3-[(3-cholamidopropyl)-dimetyl ammonio]-1-propane-sulfonate (Chaps, Fluka/VWR, Oslo, Norway) at pH 5.0] for 1 min at 22 000 rpm using a Polytron (mod. PT 1200 CL, Kinematica AG/Anders Phil AS, Dale i Sunnfjord, Norway). The extract was stored on ice for 10 min, and 1.5 mL of the homogenized sample was removed and centrifuged at 20000*g* for 30 min at 4 °C. The supernatant was then removed and stored at −80 °C until analysis. Cathepsin B, B + L, and H activity were measured according to Barrett and Kirschke (*31*). The substrates *Z*-Arg-Arg-MCA (cathepsin B), *Z*-Phe-Arg-MCA (cathepsin B + L), and Arg-MCA (cathepsin H) (Sigma, Oslo, Norway) were used. Enzyme activation was performed by incubation of 25 μL of enzyme extract with 975 μL of assay buffer (see **Table 1**) for 2 min at 30 °C, before adding 1 mL of 10 μM freshly made substrate. The optimal pH of the assay buffer was determined for cathepsins B, B + L, and H and was found to be 6.0, 6.0, and 6.6, respectively. Samples were measured in triplicate using a fluorescent spectrophotometer (Eclips, Varian/Holger, Oslo, Norway), and the excitation (λ_{ex}) and emission (λ_{em}) wavelengths were set to 380 and 460 nm, respectively. One unit of enzyme activity is defined as the amount that hydrolyses 1 mmol of substrate/min at 30 °C.

Table 1. Composition and pH of Cathepsins B, B + L, and H Assay Buffers

cathepsin B (pH 6.0)	cathepsin B + L (pH 6.0)	cathepsin H (pH 6.6)
200 mM NaH_2PO_4	200 mM NaOAc	200 mM NaH_2PO_4
2 mM EDTA	2 mM EDTA	2 mM EDTA
0.05% (w/v) Chaps	0.05% (w/v) Chaps	0.05% (w/v) Chaps
4 mM DTT[a]	4 mM DTT[a]	4 mM DTT[a]

[a] DTT was added freshly just before use. The pH giving the highest total activity was established in preliminary experiments.

2.4.2. Cathepsin D Activity Assay. The enzyme extraction buffer comprised of 1 mM EDTA and 0.2% (v/v) Tween 20 (Sigma, Oslo, Norway) in phosphate-buffered saline (PBS) buffer. The activity was measured using bovine hemoglobin (Sigma, Oslo, Norway) as the substrate (*32*). The reaction mixture consisted of 100 μL of 2.5% (w/v) hemoglobin, 50 μL of 0.2 M citrate buffer (assay buffer) at pH 2.8, and 25 μL of enzyme extract that was incubated at 37 °C for 60 min. A total of 50 μL of 15% (w/v) trichloroacetic acid (TCA; Sigma, Oslo, Norway) was added to stop the reaction, and the assay mixture was centrifuged at 20000*g* for 5 min at 20 °C. The supernatant was neutralized by adding 2% (v/v) of 10 M NaOH, and the TCA-soluble peptides in the supernatant were determined by the QuantiPro BCA assay kit (Sigma, Oslo, Norway). For the control reaction, pepstatin (Sigma, Oslo, Norway) was added before adding the enzyme extract to inhibit the enzyme reaction. Samples were measured in triplicate using a spectrophotometer (Biomate 3, Thermo spectronic, Rochester, NY) at 320 nm wavelength. One unit activity was defined as the amount of cathepsin D digesting 1 mg of hemoglobin [with bovine serum albumin (BSA; Sigma, Oslo, Norway) as the standard] in 60 min at 37 °C. The protein concentration in the enzyme extract was determined using the Bradford method after a 50-fold dilution (*33*).

2.4.3. Collagenase Activity Assay. The enzyme extraction protocol was similar to that of cathepsin D, except that the extraction buffer differed, consisting of 50 mM Tris-HCl, 100 mM NaCl, 5 mM $CaCl_2$, and 0.2% (v/v) Tween-20 (pH 7.4). Collagenase activity was determined on the basis of the method of Wunch and Heidrich (*34*), using a synthetic substrate (4-phenylazobenzyloxy-carbolyl-Pro-Leu-Gly-D-Arg) (Sigma, Oslo, Norway). Briefly, to each 1 mL assay buffer (0.1 M Tris-HCl at pH 7.1) 20 μL of 1 M $CaCl_2$ and synthetic substrate was added. For each sample, 400 μL of assay buffer and 50 μL of enzyme extract were incubated at 37 °C. After 15 min, 100 μL of 25 mM citric acid and 1 mL of ethyl acetate were added to terminate the reaction. The reaction mixture was centrifuged, and the supernatant was removed into a new tube containing 100 mg of anhydrous Na_2SO_4. The optical density of the supernatant was measured in triplicate at 562 nm wavelength using a spectrophotometer (Biomate 3, Thermo spectronic, Rochester, NY). For the blank reaction, 100 μL of 25 mM citric acid was added before the enzyme extract and incubated for 15 min. One

Table 2. Fork Length (L_F), Body Mass (g), pH, and Liquid Loss of Atlantic Halibut (Mean ± SE)

	sex	24.05.04	20.08.04	26.11.04	18.02.05	05.05.05
body mass (g)[a]	♀	1324 ± 278 (n = 13)	1906 ± 351 (n = 9)	2290 ± 236 (n = 11)	2233 ± 457 (n = 10)	2288 ± 404 (n = 13)
	♂	1126 ± 214 (n = 7)	1970 ± 287 (n = 11)	2107 ± 144 (n = 9)	1839 ± 430 (n = 10)	1690 ± 332 (n = 7)
L_F (cm)[a]	♀	47.4 ± 3.2	53.8 ± 3.4	55.9 ± 1.5	57.32 ± 3.2	58 ± 1.8
	♂	46.6 ± 2.8	53.5 ± 2.8	54.6 ± 3.1	53.8 ± 2.8	54 ± 2.7
pH[b]	♀	6.5 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1
	♂	6.5 ± 0.2	6.5 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.5 ± 0.2
liquid loss (%)	♀	ND[c]	ND	3.4 ± 0.9	9.0 ± 0.5	12.81 ± 0.7
	♂	ND	ND	4.6 ± 0.9	11.6 ± 0.7	10.8 ± 1.6

[a] See Hagen et al. (*29*). [b] See Hagen et al. (*30*). [c] ND = not determined.

unit of activity was defined as the amount of enzyme required to liberate 0.01 μmol of substrate in 15 min at pH 7.1 and 37 °C. An extinction coefficient of 0.042 for 0.01 μmol of substrate in 5 mL of ethyl acetate was used (according to Sigma). The protein concentration in the enzyme extract was determined using the Bradford method after a 50-fold dilution (*33*).

2.5. Statistics. Statistical tests were performed in the statistical packages Minitab (Release 14.20, Minitab, Inc., State College, PA) and *R* (*35*). The variables were considered separately by sex using a general linear model (GLM)

$$\text{dependent variable} = \text{sample point} + \text{sex(males or females)} + \text{fish length} \quad \text{(A)}$$

with sex and sample point as factors and fish length as a covariate. The dependent variables were transformed because of heterogeneity in the residuals of a model based on the raw data. Cathepsins B + L and H were square-root-transformed, and cathepsin D was log-transformed to comply with the model assumptions. A matrix scatter plot was constructed of all factors to identify underlying connections between factors that could affect the outcome of the chosen GAM (see below).

To investigate if enzyme type, protein, or liquid loss differed between sex for the different sampling points the following GLM was used:

$$\text{dependent variable} = \text{sample point} + \text{sex} + \text{sample point} \times \text{sex} \quad \text{(B)}$$

where sample point and sex are considered as factors and × represents an interaction. Because the data for males and females overlap in all of the sample periods, except in the last, a significant interaction between sex and sampling point will indicate that a difference between sex was present in the last sampling point.

The effect of enzyme level on protein content and liquid loss was considered using two GAMs of initial form

$$\text{protein} = \text{sex} + s(\text{cathepsin B}) + s(\text{cathepsin B} + \text{L}) + s(\text{cathepsin D}) + s(\text{cathepsin H}) + s(\text{collagenase}) \quad \text{(C)}$$

$$\text{liquid loss} = \text{sex} + s(\text{cathepsin B}) + s(\text{cathepsin B} + \text{L}) + s(\text{cathepsin D}) + s(\text{cathepsin H}) + s(\text{collagenase}) \quad \text{(D)}$$

using the library package mgcv (*36*) in *R*. *s* represents a thin-plate smooth function of each variable, with a maximum of 6 degrees of freedom. Model selection was from generalized cross-validation [gcv (*36*)]. The error structure was considered to be gamma, and an identity link function was used. Because of a risk of overfitting (unnecessarily including explanatory variables or using smooth functions with too many degrees of freedom) when using GAMs (*37*), an additional penalty was added to the smoothing (*36*).

In addition to the GAM used for analyzing the impact of enzyme activity on protein content, the relationships were also investigated using a polynomial (quadratic) regression.

3. RESULTS

Over the annual production cycle, the body mass increased by ~73 and 50% for female and male fish, respectively (p < 0.01), and the pattern of weight gain differed between the sexes (**Table 2**). Fish length showed a similar pattern (**Table 2**). In female fish, the body mass was unchanged over the winter period (November 26th−May 5th) (**Table 2**), corresponding to the period of short days when water temperature fell below the threshold for feeding (~6 °C) [Hagen et al. (*29*)]. Male fish exhibited a decline in body mass of 19.8% over the same period (**Table 2**), corresponding to the period of sexual maturation. Because sperm had been released prior to the last sample, this represented a loss of somatic tissue. The pattern of protein content change observed in the fast muscle is consistent with this tissue being a significant net contributor of amino acids during seasonal depletion in both sexes and following sexual maturation in males (**Figure 1A**). The maximum decrease in protein was up to 5.7% in females and 17.9% in males (**Figure 1A**). A GLM (model A) revealed a significant effect of sample time on protein content and a significant interaction between sex and sample period (p < 0.05, model B). In the last sample, the average protein content of fast muscle was 15.4% lower in male than female fish. The activities of cathepsins B, B + L, H, and D exhibited an approximately reciprocal pattern to that observed for fast muscle protein content over the annual cycle (parts **B**−**E** of **Figure 1**). A GLM showed that the sample point (p < 0.05, both sexes, model A) and the sample point × sex interaction (p < 0.05, model B) were significant for each cathepsin. In the last sample, cathepsin B, B + L, D, and H activities (indicated with asterisks) were 60.6, 60.2, 69.2, and 83.2% higher, respectively, in male than female fish (parts **B**−**E** of **Figure 1**, p < 0.05, model B). Regression analysis was used to further investigate the relationship between cathepsin activities and protein content (**Figure 2**). The relatively high R^2 values obtained (0.49−0.57, p < 0.001) suggest that cathepsins are likely candidates involved in the degradation of muscle protein (**Figure 2**). A GLM (model C) of the form protein = s(cathepsin B + L) + s(cathepsin D) + s(collagenase) explained 73.1% of the total variance (p values were <0.01, <0.001, and <0.01, respectively). However, such analysis should be treated with caution because cathepsin activities were highly correlated (**Figure 4**), perhaps reflecting coordinated changes in enzyme concentration with changes in lysosome abundance. Collagenase activity in fast muscle was independent of the season in female fish but was significantly lower in August than other months for male fish (p = 0.004, model A). In May 2005, the collagenase activity in male fish had increased to approximately the same levels as in May the previous year.

The WHC of the myotomal muscle, measured as liquid loss, showed a significant effect of sample time and a significant sample point × sex interaction (p = 0.45). Liquid loss was 3.4 ± 0.9 and 4.6 ± 0.9% in November (p = 0.45) compared to 9.0 ± 0.5 and 11.6 ± 0.7% (mean ± SE) in February for male and female fish, respectively (**Table 2**). The outcome of the GAM (model D) was liquid loss = s(cathepsin H), which explained 48.8% of the variance (**Figure 3**, p < 0.01). It is likely that cathepsin activities are correlated to WHC because

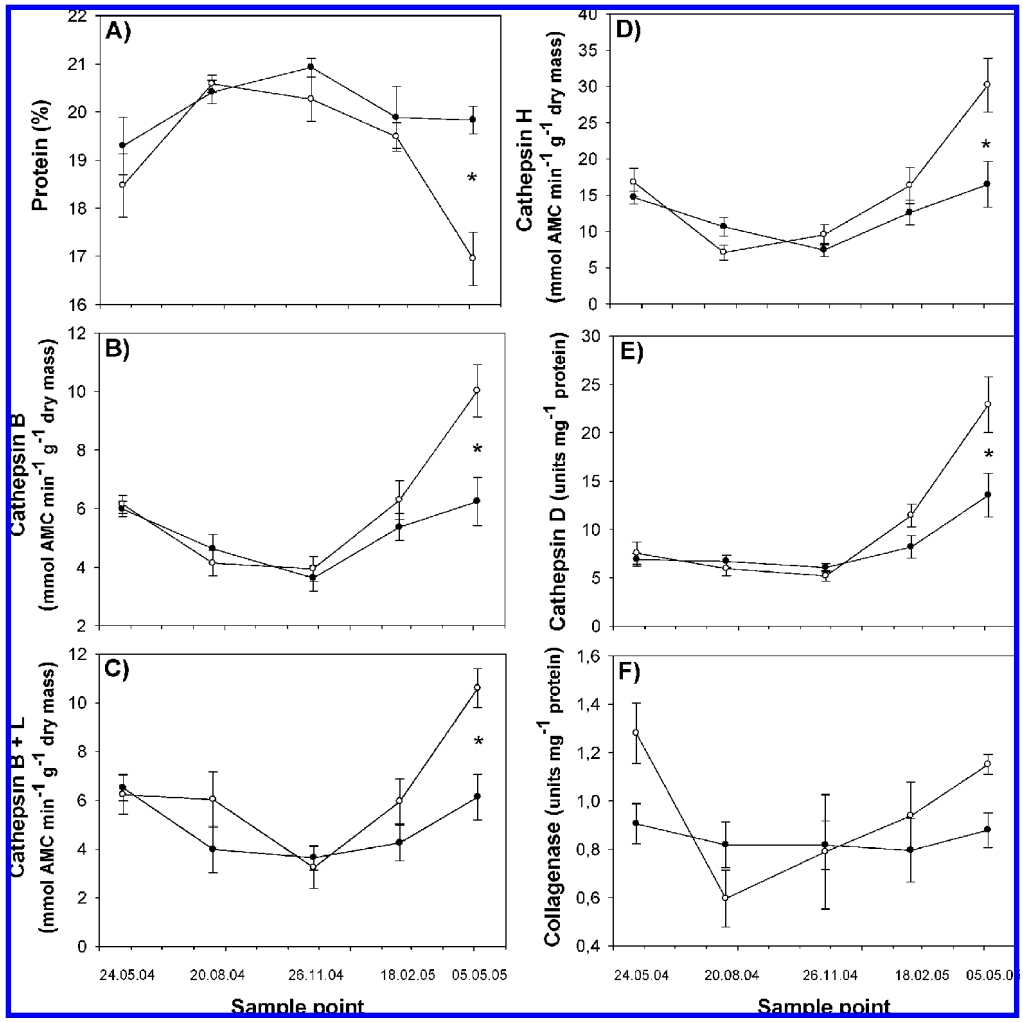

Figure 1. (A) Seasonal variation in protein content (%), (B) activity of cathepsin B, (C) cathepsin B + L, (D) cathepsin H [mmol of 7-amino-4-methylcoumarin (AMC) min^{-1} g^{-1} dry mass], (E) cathepsin D, and (F) collagenase [units (mg of protein)$^{-1}$] in male (○) and female (●) Atlantic halibut [average ± standard error (SE)]. Asterisks (∗) indicate significant differences between sex ($p < 0.05$).

of effects on protein content and distribution, although other factors are undoubtedly also important. pH did not show any sex or seasonal effect (*30*). In contrast to a previous study, no correlation between *post mortem* pH and WHC was found, perhaps because of the lack of samples with a pH < 6.3 (*9*).

4. DISCUSSION

One of the largest challenges in the grow-out phase of halibut farming is the precocious maturation of males and poor growth during the winter (both sexes) because of low water temperatures (<6 °C), which suppress feeding (*29*). In the present study, the protein content of fast myotomal muscle paralleled seasonal changes in body mass, suggesting this tissue acts as a reservoir from which amino acids are mobilized for maintenance and the generation of testis in males. The use of muscle proteins during periods of limited food supply or starvation is a well-documented phenomenon in fish species from temperate latitudes (*18, 19, 38–40*). Depletion of fast muscle protein results in a reduction in the diameter of myofibrils and myofibers, leading to a rise in water content (*38–40*), changes that are reversed with refeeding (*38–40*). The present study provides strong evidence for the involvement of cathepsins in this process (parts **A**−**C** of **Figure 2**). In agreement with the present results, Beardall and Johnston (*39*) reported fast muscle lysosomal enzyme activities increased by 70−100% during starvation in saithe (*Pollachius virens* L.). Similarly, chum salmon (*Oncorhynchus keta* L.) showed high proteolytic activity of cathepsin L during the spawning migration, and this enzyme was believed to be the most important contributor to the observed softening of the flesh (*19*). Further, cathepsin activities of fast and slow muscle peak during the spawning season of sardine (*Sardine pilchardus*) (*41*) and ayu (*Plecoglossus altivelis*) (*42*). In sea bass, the activity of cathepsins B and B + L was 29.7 and 4.7 times higher than that of bovine muscle, suggesting that these enzymes played a principal role in the *post mortem* degradation (*13*). However, the biological factors resulting in increased cathepsin activities are unknown but could be due to an increased activation and secretion of cathepsin proenzymes stored within the lysosomes, increased transcription, reduced amounts of inhibitors in the muscle, or a combination of these factors. For example, Nomata et al. (*43*) reported a decrease in specific inhibitors of calpain and trypsin activity in muscle from mature chum salmon (*Oncorhynchus keta* L.) compared to immature fish.

Myosin, actin, and tropomyosin make a significant contribution to the WHC in meat (*4, 44*). It seams likely that degradation of myofibrillar proteins will alter the WHC and possibly result in leaking of fluid from the muscle tissue, especially during processing. Of the investigated enzymes, cathepsins B + L, D, and H are known to degrade a wide range of structural muscle proteins (*12, 21, 22, 24, 25*) and

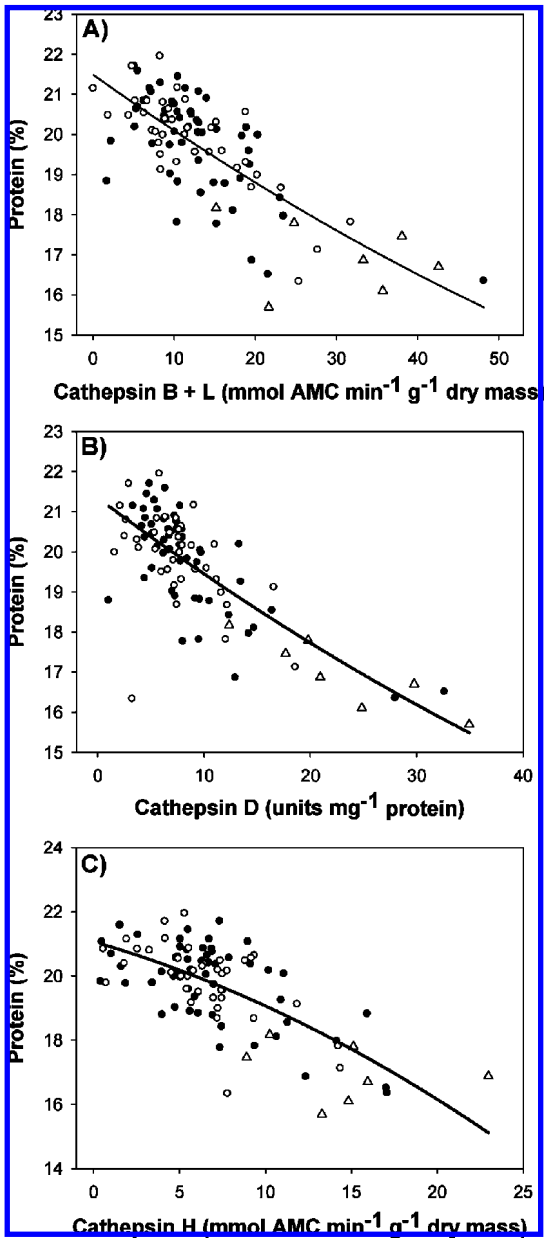

Figure 2. Polynomial regressions between protein content and the activity of (A) cathepsin B + L, (B) cathepsin D, and (C) cathepsin H; R^2 values were 0.57, 0.56, and 0.49, respectively. Open symbols illustrate males, and closed symbols illustrate females. Triangles illustrate males in the last sampling point (note the high activity level and low protein content).

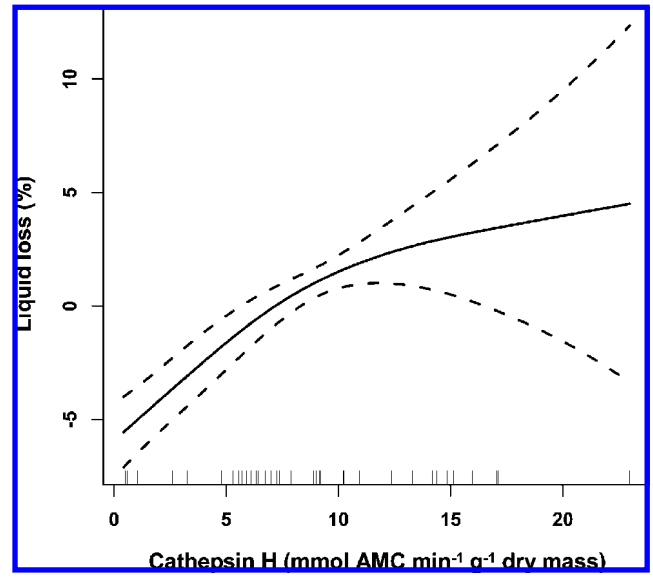

Figure 3. Graphical illustration of the outcome of the GAM liquid loss = sex + *s*(cathepsin B) + *s*(cathepsin B + L) + *s*(cathepsin D) + *s*(cathepsin H) + *s*(collagenase) (model D). The relationship between liquid loss (%) and cathepsin H explained 48.8% of the variance. Note the increase in the 95% confidence interval (dotted lines) at high enzyme activity. Zero on the *y* axis represents the average response, while the ticks on the *x* axis illustrate the distribution of the data points. Enzyme activity units are in mmol of AMC min^{-1} g^{-1} dry mass.

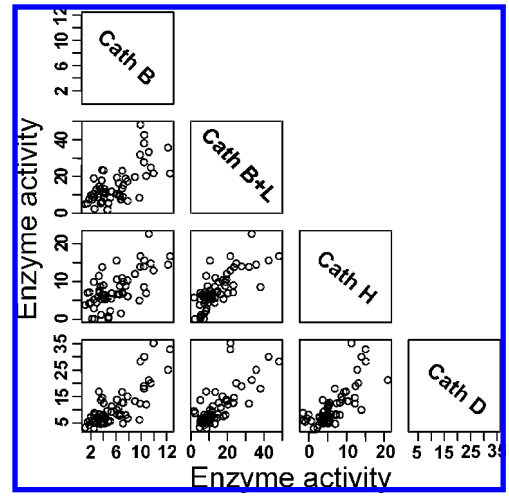

Figure 4. Matrix scatter plots illustrating the connection between the cathepsins B, B + L, H, (mmol of AMC min^{-1} g^{-1} dry mass) and D [units (mg of protein)$^{-1}$].

are therefore potential candidates to influence the protein content and hence liquid loss of fish muscle (**Figures 2** and **3**). The relationship between liquid loss and cathepsin H activity is nonlinear and approaches asymptotic values (**Figure 3**). Although cathepsin H showed the strongest correlation with liquid loss, the interactions between variables complicates the analysis (**Figure 4**). It is most likely that there are synergic effects of several proteolytic enzymes on muscle protein breakdown (parts **A**−**C** of **Figure 2**) (*13, 14*) in combination with other factors eventually affecting liquid loss (*6, 7, 9, 10*). Other candidate proteolytic enzymes involved are the calpain family, which are also known to mobilize myofibrillar proteins in fish (*13, 14, 20*).

It is concluded that the seasonal changes in liquid loss observed in commercially farmed Atlantic halibut (**Table 2**) are in broad agreement with published results from small-scale experiments (*8, 9*), indicating that it is a robust finding. Further, it is documented that cathepsins have a significant impact on the protein content and hence liquid loss in Atlantic halibut muscle and are therefore key enzymes in catabolic processes. The present results support previous conclusions regarding the timing of the harvesting of Atlantic halibut (*30*). From a quality point of view, the fish should be harvested in the fall or early winter when the liquid loss, nutritional value (*29*), and texture (*30*) are favorable, increasing consumer acceptability and reducing any downgrading losses during secondary processing (e.g., drip loss). If the fish is harvested during the spring, it is likely to reduce the shelf life of the product because of the high cathepsin activity (parts **B**−**E** of **Figure 1**), which will increase the rate of muscle disintegration during storage (*45*). However, to establish if low liquid loss, firm texture, and a good nutritional

value of halibut are synonymous with optimal eating quality, a sensory test needs to be conducted.

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