ASSESSING AND MINIMIZING FILLET DISCOLOURATION IN COMMERCIALLY HARVESTED YELLOWTAIL FLOUNDER (*Limanda ferruginea*)

JESSICA L. KENNEY

Assessing and minimizing fillet discolouration in commercially harvested yellowtail flounder (*Limanda ferruginea*)

by

© Jessica L. Kenney

A Thesis submitted to the School of

Graduate Studies in partial fulfilment of the

requirements for the degree of Masters of Science

Biology, Faculty of Science
Memorial University of Newfoundland

March, 2012

St. John's Newfoundland

Abstract

In the commercial yellowtail flounder (*Limanda ferruginea*) industry, the choice of operational techniques used to harvest and process this protein can dramatically affect the quality of the end product to consumers. In the case of whole fillet products, one of the most common challenges is fillet discolouration (bruising), inflicted through harvesting and processing techniques. This thesis aimed to develop an objective assessment technique for bruising, as well as characterize the bruising patterns that are currently seen in commercially harvested yellowtail flounder (*Limanda ferruginea*). It also aimed to assess the effectiveness of bleed method, bleed time and freezer orientation to give recommendations on optimal processing techniques for minimal bruising. Major findings include that bruising occurs equally frequently on both fillets, and on both the inside and outside of the fillets. Bruises were located non-randomly, occurring predominantly at the anterior region of the fillets. Bruise method and bleed method had no significant effect on overall bruising, but fish frozen in a horizontal plate freezer had less bruising than those frozen in a vertical plate freezer.

Acknowledgements

I would like to thank Dr. Paul Winger and Ms. Heather Manual for being such excellent supervisors. Their guidance and input has been invaluable, especially during setbacks and technical difficulties. This project as taught me a lot about problem solving and experimental design, through which Dr. Winger and Ms. Manual's support has been very much appreciated. Also, thank you to my thesis committee members Mr. Randy Gillespie and Ian Fleming for their council and editing.

I would like to thank Mark Santos, Taufiqur Rahman, Kurt Gamperl, Brian Gillet, Chris Dawe, Tracy Granter, Jason Nichols, Kevin Carroll, Michelle Thompson and Kim Snelgrove for their technical assistance, as well as OCI and the crew of the *F/V Aqviq*. A special thank you to Melanie Underwood for all of her time and energy spent aboard the *Aqviq* collecting and maintaining live yellowtail for me. I am also grateful to several individuals for their discussion and advice on the project, including Emma Posluns, Kelly Moret, Scott Grant, Rennie Sullivan, George Legge, Tara Perry, Harold DeLouche, Georgina Bishop, and Philip Walsh. Also, a huge thank you to Claudene Hartery for always keeping me organized and helping me with paperwork.

Finally, I would like to thank my parents, Chris and Sue, as well as my sisters, Kelly and Kathryn, for always being there for me, and for encouraging me every step of the way. Their support and sense of humour have made me the person that I am today. I would like to say thank you to Katie Sullivan for being my best friend, and for moving to

Newfoundland with me. Also, thank you to the Hennessey family for being a second home for me for the past year.

The project was funded by Ocean Choice International, the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Centre for Fisheries Innovation (CCFI), and the Department of Fisheries and Aquaculture (DFA), Government of Newfoundland.

Table of Contents

List of Tables

List of Figures

Co-authorship Statement

The author of this thesis designed the experiments, collected/organized all of the data, analysed and wrote all of the subsequent manuscripts. Paul D. Winger and Heather Manual contributed significantly to research proposals, experimental designs, and discussion of ideas and provided editorial reviews of all chapters. Mark Santos contributed significantly to the experimental design and data collection for Chapter 2. Taufiqur Rahman was integral to the design of the computer program and methods used in Chapter 2. Mark Santos also made substantial contributions to the experimental design and data collection for Chapter 3.

Taufiqur Rahman, Paul Winger and Heather Manuel are second, third and fourth authors for Chapter 2, respectively.

Mark Santos, Dr. Paul Winger and Dr. Heather Manuel are second, third and fourth authors for Chapter 3, respectively.

Chapter 1. Introduction and Overview

1.1 National and Global Fisheries

In 2010, the Food and Agriculture Organization (FAO) of the United Nations reported that 32 percent of fish stocks worldwide were overexploited, depleted, or recovering, compared to 10 percent in 1974. In 2008, a total of 142 million tonnes of fish were supplied to the world's population, 90 million tonnes of which was supplied by capture fisheries (FAO, 2010). Of this total, 115 million tonnes were used for human food, providing 4.5 billion people worldwide with as much as 20 percent of their intake of animal protein (FAO, 2010). Along with being a food staple, the primary fish sector and associated secondary activities also provided a source of income and livelihood for approximately 180 million people globally, up 167 percent since 1974. Including the dependents of those employed in the fisheries, it is estimated that 540 million people, 8 percent of the world's population, were directly dependent on capture fisheries (FAO, 2010).

China is by far the leading producer of fish with 47.5 million tonnes of fish in 2008, 14.8 million of which were from capture fisheries (FAO, 2010). Other major capture fisheries included Peru (7.4 million tonnes), Indonesia (5.0 million tonnes), the United States of America (4.3 million tons) and Japan (4.2 million tonnes). Major exporters around the world include China (USD 10.1 billion, 14.3 percent of production), Norway (USD 6.9 billion, 6.6 percent of production), Thailand (USD 6.5 billion, 4.9 percent of production), Viet Nam (USD 4.6 billions, 4.7 percent of landings) and the United States (USD 4.5 billion, or18.7 percent of production). Canada, in comparison, exported USD 3.7 billion

in 2008, approximately 5.0 percent of seafood produced (FAO, 2010). Leading importers worldwide include Japan, the United States, Spain, France and Italy (FAO, 2010).

In 2010, Canada produced a total of 939,460 metric tons of seafood (Department of Fisheries and Oceans, 2011). Many major fish stocks in Canada have rapidly declined in recent years, some even collapsing entirely in the 1990s (Figure 1.1). Changes in the harvest levels occur in response to changes to complex marine ecosystems, as well as to human activity. Canadian fisheries have somewhat recovered in recent years, and in 2009, Canada's fisheries sector landed about $1.6 billion worth of fish, with an export income of about $3.6 billion.

In 2010, 324,610 tonnes of seafood were produced in Newfoundland, 35 percent of Canada's total landings (totaling approximately $439 million, up 3.7 percent from 2009; Department of Fisheries and Aquaculture, 2011). Newfoundland is a major exporter of seafood products, primarily to the United States, China, the United Kingdom and Denmark (Figure 1.2). Of total Newfoundland landings, 11 percent, or 39,663 tonnes was groundfish (21 percent of total Canada groundfish production) (Figure 1.3), and of that, 10,885 tonnes were flatfish (27 percent of Newfoundland groundfish production, and 60 percent of all Canadian flatfish production; Department of Fisheries and Aquaculture, 2011). In 2010, yellowtail flounder landings rose 47.7 percent from 2009, composing 6 percent of all landed groundfish value in Newfoundland (DFA, 2011). Interestingly, contrary to increases in landings, yellowtail production decreased 12.7 percent in the same time period. This is due to the increasing globalization of the fisheries value chain - the increasing practice of harvesters outsourcing production to developing countries, such as China. The extent of the practice depends on species, desired product, and the cost of

labour and transportation (FAO, 2010). In 2010, provincial capture fisheries employed 21,142 individuals, a 5.3 percent decrease from 2009 due to production out-sourcing, as well as an aging workforce, out-migration and competing employment opportunities (DFA, 2011).

1.2 Newfoundland Yellowtail Flounder

The yellowtail flounder, *Limanda ferruginea*, is a right-eyed flatfish that inhabits the coastal areas and continental shelf from Labrador to Chesapeake Bay (Bigelow and Shroeder, 1953), with large commercial stocks located on the Grand Bank and Georges Bank (Murawksi *et al* 1997). The commercial flounder fishing industry is targeted nearly exclusively by Canadian vessels, and has been an extremely integral part to Newfoundland's economy since the 1960's (Walsh, 1991) and has since fluctuated widely. The industry experienced its highest recorded catches (approaching 40,000 metric tons) in the 1970s, followed by decline in the 1980s. Total collapse in the early 1990s led to a yellowtail moratorium from 1994-1997, and moderate recovery in recent years (Brodie *et al.*, 1998; Walsh *et al.*, 2004, and Maddock Parsons *et al.*, 2011) (Figure 1.4). In 2006, unexpectedly low landings (177 metric tons), the result of corporate restructuring and labour disputes, led to the temporary closure of the Fisheries Products International (FPI) yellowtail processing plant in Marystown. The plant was re-opened in 2007 under the new ownership of Oceans Choice International (OCI), the largest employer in the region. Between 2000 and 2011, the plant was the primary location for flounder processing, employing between 200 and 300 people (Wellman, 2009), however the facility closed in late 2011 due to poor economic viability. Approximately 13,000

metric tons of flounder, three quarters of Atlantic Canada's landings, were harvested in Newfoundland alone in 2008, totaling nearly $8,000,000 of commercial revenue (Department of Fisheries and Oceans, 2008). Lower yellowtail landings, due to a decrease in fishing effort in response to lower market prices, contributed to an overall decrease in Newfoundland's groundfish catch by 13 percent, and a corresponding dollar value decrease by 25 percent (Department of Finance of Newfoundland, 2010).

Due to its nature, the capture fishery for yellowtail flounder is extremely energy intensive. The species is harvested by offshore factory freezer trawlers (Figure 1.5) using specially engineered mobile bottom trawls, which are towed over the seabed at speeds between two and three knots (Figure 1.6). The capture process involves a complex sequence of herding and guiding fish toward the mouth of a net, where they then swim at speeds exceeding their maximum sustained swimming speeds (less than 30 seconds) before exhausting and falling back into the net (see review by Winger *et al.* 2010). Once inside the net, the fish are guided by netting panels toward the codend where they collect until the end of the tow. Small fish escape through the mesh (known as mesh selectivity) while larger and more marketable fish are retained. Tow duration varies depending on the Captain's discretion, but typically ranges between one and four hours. Once below deck, fish are then sorted according to size, and gutted manually. Gutted fish then pass through a tank of sea water (bleed tank) where excess blood residue is rinsed from the body over a period of several minutes (bleed time). Once bled, fish are flash frozen in vertical or horizontal plate freezers and kept frozen until return to port.

Transporting the product from the water to the consumer expends large amounts of energy and requires hundreds of trained employees. Because of this, ensuring that harvesting and processing techniques are efficient and sustainable is of significant interest to the industry. Numerous factors currently make competing in the industry difficult, including an increase in fuel costs, the strengthening of Canadian currency compared to the US dollar, the expansion of the manufacturing sector in China, the union of global seafood buyers and producers, an aging workforce and the emergence of similar competing products on the market (ex. Alaskan sole). In response to these challenges, operational efficiency in both harvesting and processing sectors is an absolute necessity.

1.3 Fillet Discolouration

Minimizing losses throughout every step of the harvesting and processing operations is critical to maximizing value from the yellowtail fishery. One particular area where losses sometimes occur is the trimming of fillets due to unintentional damage and discolouration. Prior to being hauled in, crowding in the net, meshing (fish becoming stuck in mesh), and barotrauma are all potential sources of fillet damage. It has been shown that trawled fish have more discolouration than fish caught by longline (Rotabakk *et al.*, 2011). Handling practices onboard a vessel have been shown to affect flesh discolouration in fish as well (Huss, 1995). Discolouration is caused by physical or physiological trauma that causes blood vessels to rupture and blood residue to pool is certain locations (Figure 1.7). Discolouration has not been shown to affect the taste of fillets, but is disadvantageous for a number of reasons. Firstly, blood residue within the fillets is a potential source for lipid oxidation and hydrolysis of blood cells, leading to

proteolysis of the muscles, accelerating flesh softening (spoilage) *post* mortem (Ando *et al.*, 1999; Grunwald and Richards 2006). Secondly, discolouration detracts from retail value because it is visually unappealing to consumers and thus must be trimmed away prior to packaging and distribution (Francis, 1995; Robb *et al.*, 2003; Roth *et al.*, 2005; Olsen *et al.*, 2006). The trimming process leads to a decrease in fillet size, and consequently a loss in total yield and profit. For this reason, any practices that could lead to a decrease in fillet discolouration would be extremely valuable to the commercial fishing industry.

Little research has been done on fillet discoloration in yellowtail flounder, but other species have been looked at in both commercial and recreational fisheries. Assessment in recreational fisheries has tended to occur mainly in angling tournaments, and has aimed to minimize post-angling mortality and damage. Researchers in recreational angling environments tend to use visual assessment, classifying discolouration in terms of none, moderate or severe (e.g., Morrissey *et al.*, 2005). Visual assessment and grading have been used in commercially valuable fisheries for both quantitatively counting blood spots on salmon fillets (Roth *et al.*, 2009), and qualitatively describing discoloured fillets of Atlantic salmon, rainbow trout, Atlantic cod, and haddock in terms of severity (Schill and Elle, 2000; Akse and Joensen, 2004; Roth *et al.*, 2009; Digre *et al.*, 2010; Rotabakk *et al.* 2011). Visual assessment has been facilitated by the use of a transparent plastic grid to measure discoloured areas on cod fillets (Margeirsson *et al.*, 2006). The benefits of visual assessment of fillet discolouration include low cost, and simplicity.

One of the drawbacks of visually assessing fillet discolouration is that it is highly subjective. It is difficult to ensure that assessments are consistent both between researchers and between trials, which makes it difficult to make meaningful comparisons of the results of different studies. To overcome this challenge, some researchers have applied photography, computer image analysis and spectroscopy to characterize discolouration (and other defects, such as nematodes) in herring (Hamre *et al.*, 2003), turbot (Roth *et al.*, 2007), Atlantic cod (Heia *et al.*, 2007; Rotabakk *et al.* 2011) and Atlantic and Chinook salmon (Zydlewski *et al.*, 2008; Erikson *et al.*, 2010). The benefits of image analysis include objectivity, as well as the ability to create a reliable baseline from which to compare later research to detect the impact of changes to processing technique.

1.4 Overview

Collaborative research between Memorial University and Ocean Choice International was initiated in 2009 to address several harvesting and processing related operational challenges. The purpose of this thesis in particular was to characterize the discolouration patterns that are currently experienced in commercially harvested yellowtail flounder, investigate their potential causes, and explore possible changes to processing techniques to minimize the problem.

The first experimental chapter (Chapter 2) investigates the quantification of bruising patterns in commercially harvested yellowtail flounder (*Limanda ferruginea)*. This project aimed to develop an assessment technique for bruising, as well as characterize the

bruising patterns that are currently seen in commercially harvested yellowtail. Harvested and gutted yellowtail were filleted and skinned, photographed and digitally analyzed by a computer program developed for the study, and then manually assessed for bruising. During manual assessment, fish were measured for length and weight, and bruised regions were trimmed out and weighed. Fish weight and condition factor were shown to be correlated to bruise weight. Bruise area was correlated with bruise weight, but with no other physical characteristics. Left and right fillets were equally bruised, as were the inside and outside of the fillets. As hypothesized, bruises were found to be located non-randomly, occurring predominantly in the anterior region of the fillet and increasingly with distance from the centre of the fillet. The outcomes revealed information about the cause of discolouration, as well as the development of a new objective assessment protocol for future studies.

The second experimental chapter (Chapter 3) investigates the effect of processing techniques on fillet discolouration in commercially harvested yellowtail flounder. Three slaughter methods (gutting, bobtail and gill slit), three bleed times (5, 10 and 20 minutes), and freezer orientation (vertical and horizontal) were investigated to determine their effect on internal bruising. Based on previous research, it was predicted that bruising would be less in fish slaughtered by gutting than those by gill slitting and bobtailing. It was also hypothesized that bruising would decrease with increased time spent in the bleed tank. Lastly, it was expected that bruising would be less in fish frozen in vertical plate freezers due to the increased ease with which residual blood may drain from the body. It was found that fish are heavier overall in November than in February, and that when fish

are lighter, weight is a better predictor of internal bruise area than when they are heavier. Bleed method and bleed time had no significant effect on bruise area. Fish frozen in the horizontal plate freezer had less bruising than those frozen in the vertical plate freezer.

The thesis ends with a summary chapter in which the results are discussed along with benefits and limitations of the approach, and speculation on the direction for future research needs is presented.

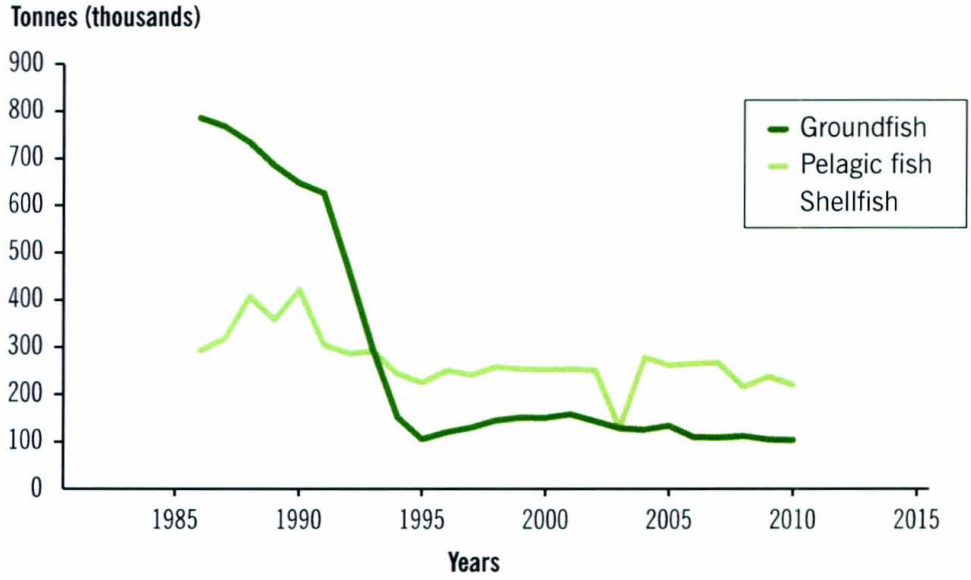


Figure 1.1 Commercial harvests of some of Atlantic Canada's fish stocks since 1986 (*from Office of the Auditor General, 2011*). Note: Data for 2010 is preliminary.

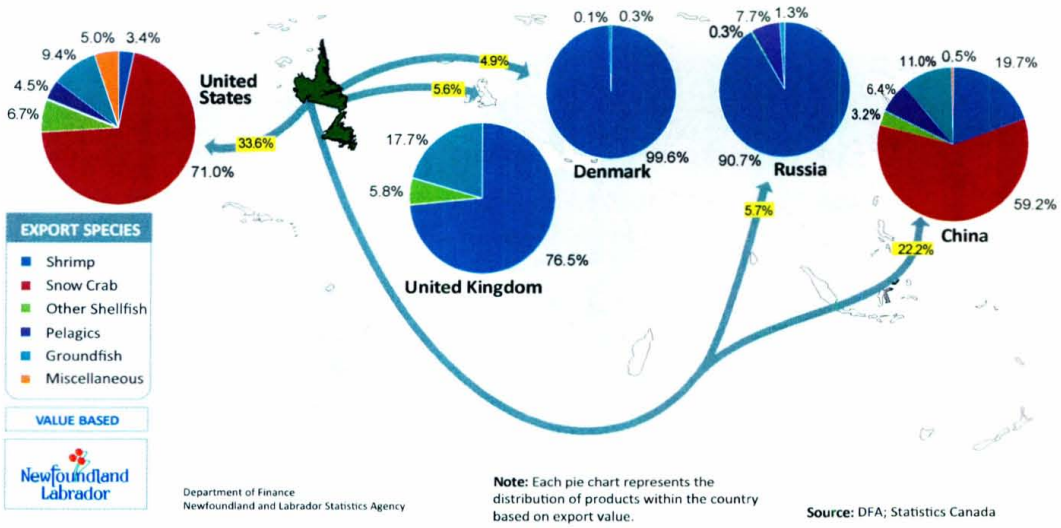
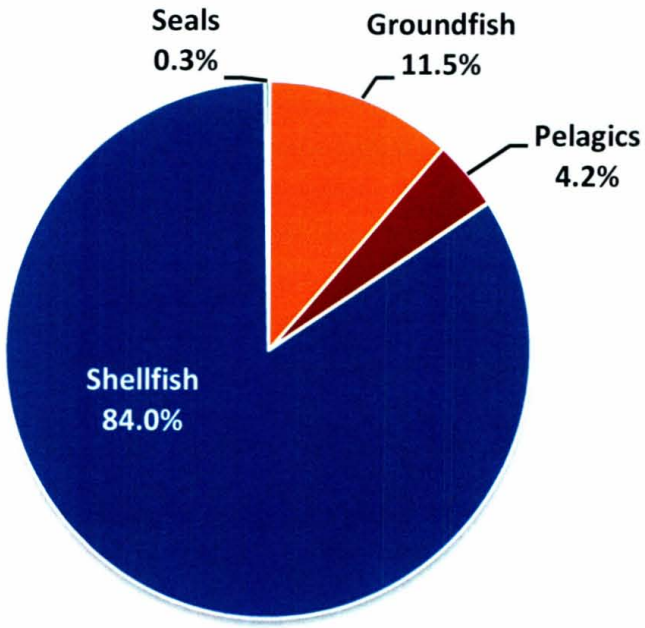


Figure 1.2 Top five world markets for Newfoundland and Labrador seafood products, 2010 (*from DFA, 2011*).



Note: Total landed value = $439 million.

Source: DFO; DFA

Figure 1.3 Capture fisheries landed value by species group, 2010 (*from DFA, 2011*).

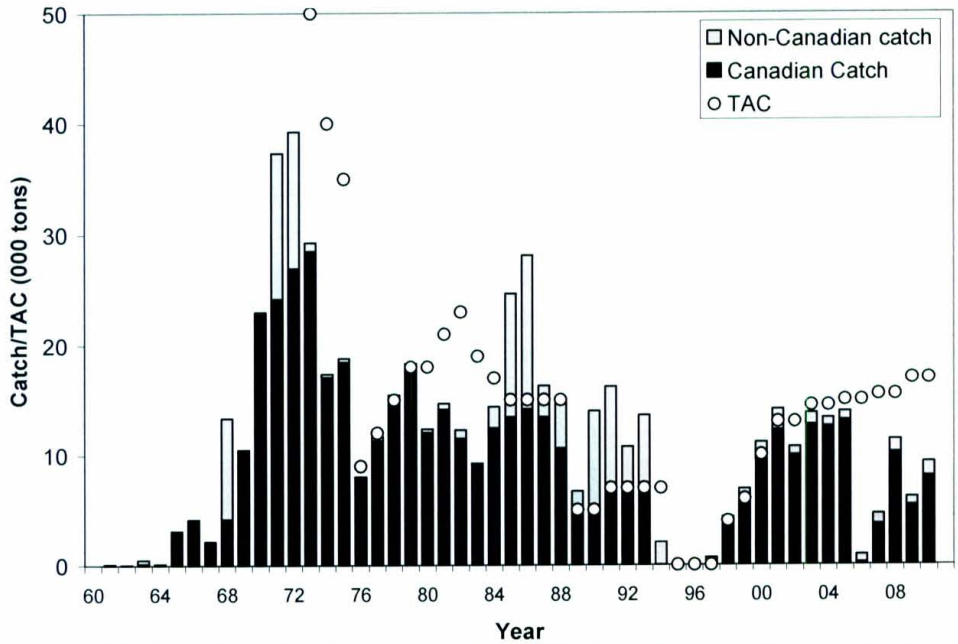


Figure 1.4 Catch and total allowable catch (TAC) for yellowtail flounder in Northwest Atlantic Fisheries Organization (NAFO) Div. 3LNO (*from Maddock Parsons et al., 2011*).

Figure 1.5 Ocean Choice International factory freezer trawler fleet, the Aqviq (upper photo), the Kinguk (middle photo) and the Atlantic Viking (lower photo).

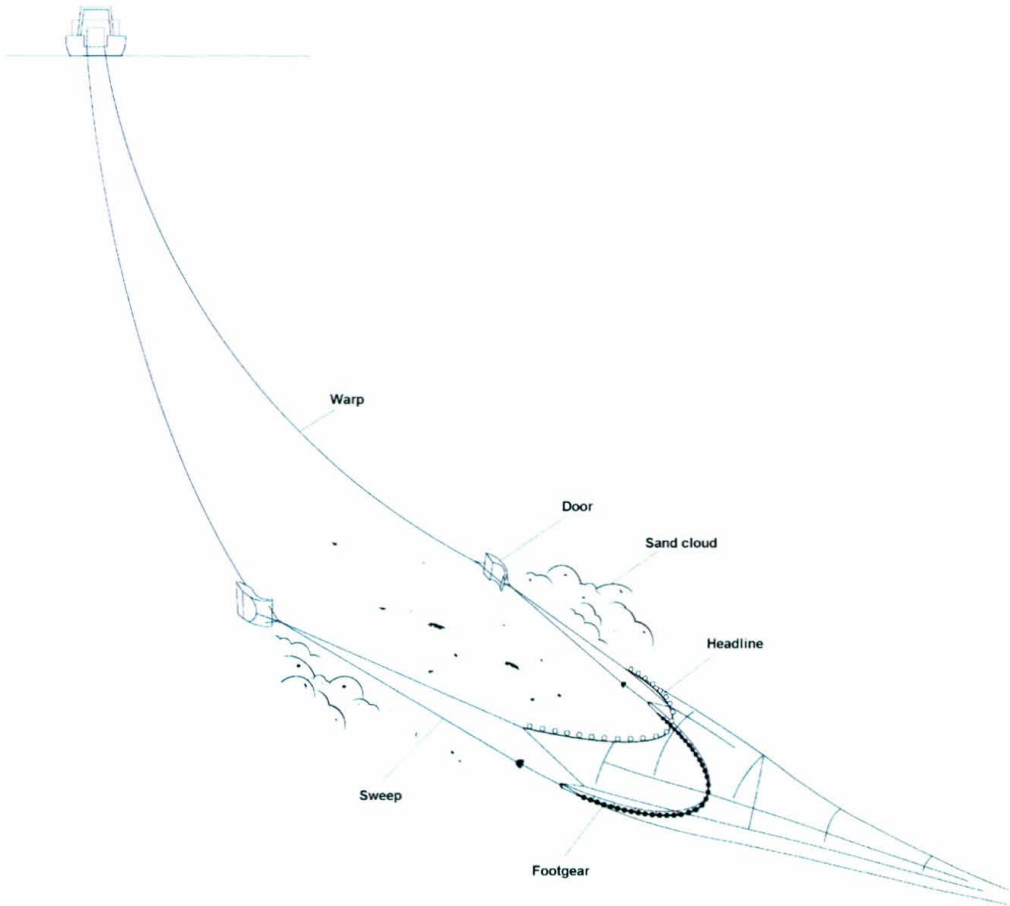


Figure 1.6 Schematic drawing of a complete bottom trawl system. Design, shape and size of individual components vary depending on the fisher and operation (adopted from Winger *et al.*, 2010).

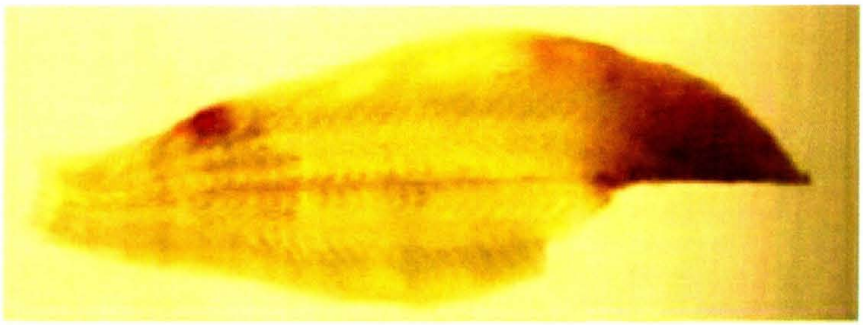


Figure 1.7 Typical RGB (red, green and blue channel) image of a fillet. Darkened areas are considered to be discoloured, or bruised.

Chapter 2. Quantification of bruising patterns in commercially harvested yellowtail flounder (*Limanda ferruginea*).

2.1 Introduction

Total global production for marine capture fisheries currently fluctuates between 80-90 million tonnes annually (FAO, 2010). The choice of operational techniques used to harvest and process this protein can dramatically affect the quality of the end product to consumers. In the case of whole fillet products, one of the most common challenges is fillet discolouration. Prior to being hauled in, crowding in the net, meshing (fish becoming stuck in mesh), and barotrauma are all potential sources of fillet damage. Lipid oxidation of fresh fillets has been shown to affect fillet discolouration (Ruff *et al*., 2003), as have handling practices on and below a ship's deck (Huss, 1995). Discolouration is caused when physical or physiological trauma causes blood vessels to rupture and blood residue to pool in certain locations. Though this discolouration has not been shown to affect the taste of fillets (Roth *et al.*, 2007), it is disadvantageous for a number of reasons. First, blood residue within the fillets is a potential source for lipid oxidation and hydrolysis of blood cells, leading to proteolysis of the muscles, accelerating flesh softening (spoilage) *post* mortem (Ando *et al.*, 1999; Grunwald and Richards 2006). Second, from a consumer standpoint it is visually unappealing, detracting from retail value, and thus must be trimmed away prior to packaging and distribution (Robb *et al.*, 2003; Roth *et al.*, 2005; Olsen *et al.*, 2006). The trimming process leads to a decrease in fillet weight, and consequently a loss in total yield and profit. For this reason, any

practices that could lead to a decrease in fillet discolouration would be extremely valuable to the commercial fishing industry.

Interestingly, fillet discolouration is a defect that is very common to yellowtail flounder, but much less so to the American plaice (*Hippoglossoides platessoides*) yet the two species are similar in size and shape, and are harvested and processed in much the same way. Different flatfish within the right-eyed flounder family Pleuronectidae have been shown to have differences in blood volume and hemoglobin concentration, for example European plaice (*Pleuronectes* platessa) have less than half the blood volume of, and 30% lower hemoglobin concentrations than European flounder (*Platichthys flesus*) (Buddenbrock *et al.*, 1934). This presents the possibility that increased fillet bruising in yellowtail may be attributed partially to physiological factors.

In order to assess the effects of changes made to processing techniques, a better understanding of what the current patterns of discolouration look like and the development of a standard assessment technique are necessary. Previous research on bruise assessment and quantification has been conducted, mainly in the recreational fisheries, but also, to a lesser extent, in the commercial fisheries. Assessment in recreational fisheries has tended to be mainly in the interest of live-release and minimizing mortalities in recreational angling tournaments. Assessment has been mostly visual, with researchers often classifying bruises in terms of none, moderate or severe (e.g., Morrissey *et al.*, 2005). Visual assessment and grading have been used in commercial fisheries for both qualitatively describing discoloured fillets of rainbow trout,

Atlantic cod, and haddock (Schill and Elle, 2000; Digre *et al.*, 2010), occasionally with the aid of photographs depicting examples of each severity level or quantitatively by counting blood spots on salmon fillets (Roth *et al.*, 2009). In some instances, the number of red blood cells contained in stained sections of fish muscle has been measured using a light microscope (Ando *et al.,* 1999). Visual assessment has been improved by the use of a transparent plastic grid to better estimate discoloured areas on cod fillets (Margeirsson *et al.,* 2006). Visual assessment of fillet discolouration has a number of benefits including its low cost, and the fact that it requires little or no complex technology, which means it can be readily performed both in the laboratory and on a vessel at sea.

One of the drawbacks of visually assessing fillet discolouration is that it can be extremely subjective. It is difficult to ensure that assessments are consistent both between researchers and between trials, which makes it difficult to compare results from one study to the next. To overcome this challenge, some researchers have conducted chemical analysis of fish muscle (Grunwald and Richards, 2006), or applied photography and computer analysis to assess discolouration. Spectroscopy and/or image analysis software has been used to characterize discolouration (and other defects, such as nematodes) in herring (Hamre *et al.*, 2003), turbot (Roth *et al.*, 2007), Atlantic cod (Heia *et al.*, 2007) and Atlantic and Chinook salmon (Zydlewski *et al.*, 2008; Erikson *et al.*, 2010). The benefits of image analysis include objectivity, as well as the ability to create a reliable baseline from which to compare later research to detect the impact of changes to processing technique.

The purpose of this study was to develop a method to objectively measure and characterize the size and location of discolouration patterns that are currently observed in commercially harvested yellowtail flounder. To accomplish this, an image processing program was devised to analyze fillet discolouration patterns. Total discoloured area and percent fillet coverage were measured and compared to total bruise weight and percent bruise weight attained from manual assessment. It is expected that the outcome of this analysis will reveal information about current discolouration patterns, as well as create an assessment protocol for future studies.

2.2 Methods and materials

2.2.1 Frozen Fish Acquisition

Yellowtail flounder were harvested by Ocean Choice International during a commercial fishing trip in June 2009 aboard the vessel *F/V Aqviq*. The fish were processed (i.e. gutted and bled) according to standard processing protocols and flash frozen in 23kg blocks. They were then transported by truck from the processing facility in Marystown, NL to the Fisheries and Marine Institute in St. John's, NL, where it remained frozen at -30 degrees C.

2.2.2 Manual Assessment for Bruise Weight

Yellowtail flounder were removed from the freezer in September 2010 and thawed overnight for filleting and bruise evaluation in the Pilot Processing Plant at the Fisheries and Marine Institute. Due to the fillets being frozen for 15 months, flesh quality concerns were raised. It is known that exposure to light while frozen tends to affect the oxidation,

and as a result, the quality and colour of the flesh. Fish used for this study were flash frozen whole with skin intact, and stored in the dark, so for the purposes of this project it is assumed that freeze duration did not affect flesh quality. A total of 157 individual fish were sexed, measured for total length (± 1mm) and weighed (± 0.1g). Fish were then filleted and skinned, and the fillets weighed. Both the outside and the inside of each fillet were photographed (Figure 2.1a and 2.1b) on a light table to facilitate bruise detection. Bruises were visually identified as regions of the fillet that were more reddish in colour than the surrounding fillet. Visible bruises were then manually removed with a filleting knife and weighed. To deal with the error associated with the subjective nature of this method, the same technician assessed and trimmed each fillet in accordance with traditional processing plant standards.

2.2.3 Digital Image Analysis for Bruise Area

GEOMETRIC CHARACTERIZATION OF THE FILLETS

The input images featured two fillets in each image with very little contrast between the background and the fillets. Figure 2.2 shows a typical input image. Input images were RGB, containing red, blue and green colour channels. From an image processing point of view, this lack of contrast is not ideal as it is generally difficult to segment the object of interest from the background. In order to overcome this difficulty, the contrasts between the fillets and the background, available in the three individual colour channels (red, green and blue), were taken into account (Figure 2.3a). For most of the images the blue channel exhibited the most contrast. Therefore, the blue channel was chosen as the primary input for the segmentation task. Image segmentation classifies the pixels of the

input image into two categories (object of interest and background); i.e., whether a pixel belongs to the object class or background class. A suitable gray level threshold must be determined for the segmentation task. This threshold should categorize each pixel of the input image into two groups (background and fillet) in a robust manner. Though the lighting conditions can be safely assumed to be uniform for the entire data set, a static threshold generally performs poorly and often yields unreliable results. A more robust alternative is Otsu's method, where the gray level histogram of the image is taken into account to determine an optimal threshold. Otsu's method finds the optimal threshold (Otsu, 1979) that separates the pixels into two classes in such a way that their intra-class variance is minimal. Figure 2.3b shows the gray level histograms of the three color channels of the RGB image in Figure 2.2. The gray level thresholds found by Otsu's method for the red, green and blue color channels of the image in Figure 2 were 199, 166 and 98 respectively. For fillet and background segmentation, however, only the blue channel was used and the pixels were classified using the Formula in Equation 1. In Equation 1, $I_b(i, j)$ is the gray level value of the pixel from the i-th row and j-th column in the blue channel of the input image, T_b is the threshold determined by Otsu's method from the blue channel and $B(i, j)$ is the binary value of the pixel from the i-th row and j-th column of the segmented image.

$$\boldsymbol{B(i,j)} = \begin{cases} \mathbf{1}, & \boldsymbol{if\ I_b x < T_b} \\ \mathbf{0}, & \boldsymbol{if\ I_b \geq T_b} \end{cases} \quad (1)$$

An example of the segmentation process is shown in Figure 2.4, with the image segmented into blobs and background, as per standard blob analysis. As seen in the image, the segmentation process typically identified several extraneous regions as fillet in addition to the actual fillet regions. In order to filter out these regions, the fact that the fillets are the two largest regions in the image was exploited. A rigorous testing of the segmentation algorithm revealed that for most of the images, this assumption worked very well. Once the fillets were segmented, a crack code contour tracking algorithm (Wagenknecht, 2007) was employed to determine the following geometric properties of the fillets: area, perimeter, centroid, major and minor axes, vertices of the rectangle that bounds an individual fillet with minimum area. The algorithm also stored all the contour points for future reference.

IDENTIFICATION AND LOCALIZATION OF BRUISES IN THE FILLETS

In absence of any specialized lighting (e.g., UV), the image processing program depended on the chromatic properties of the bruises to identify and localize them. It was observed that the bruised regions absorb comparatively more light. This interesting phenomenon is demonstrated in Figure 2.5 where the colour channels were inverted using the formula in Equation 2.

$$I_x^{`} = 255 - I_x(i,j) \quad (2)$$

In Equation 2, $I_x(i, j)$ is the gray value of any colour channel from the i-th row and j-th column, and $I'_x(i, j)$ is the corresponding inverted value of the corresponding pixel position. In the inverted images of the different colour channels (Figure 2.5), the bruised regions appeared to be whiter than the rest of the image. However, this distinction was

not very prominent for the inverted blue channel. As a result, only the red and green channels were considered for identification and localization of the bruises. The formula in Equation 3 was then used to isolate the probable pixels that may belong to a bruised region.

$$B_b(i,j) = \begin{cases} 1, & if\ I_g^{`}(i,j) > T_g,\ I_r^{`}(i,j) > T_r \\ 0, & if\ I_g^{`}(i,j) \leq T_g,\ I_r^{`}(i,j) \leq T_r \end{cases}$$

(3)

The probable pixels that may belong to a bruised region were identified by employing the formula in Equation 3 and an example of the resultant image is shown in Figure 2.6.

Once all the extraneous probable bruise regions were filtered out, a contour tracking algorithm was employed to determine the geometric properties of the bruised regions. See Figure 2.7 for example. From this data, fillet areas and bruise areas were calculated, as well as the exact position of each bruise relative to the fillet's geometric centroid (the point at which the major and minor axis of the fillet intersect), computed by the program.

2.2.4 Analysis

The relationship between body length and weight was calculated according to the equation $W = aL^b$, where W = fish weight (g), L = fish length (mm), b (the allometric coefficient) is equal to the slope and a is equal to the intercept of the regression line of logL x logW. Condition factor was calculated as $100W/L^3$. Differences in fish length, fish weight, fillet weight and condition factor between males and females were compared

using a one-way ANOVA. The relationship between fish length, fish weight, fillet weight and condition factor and bruise weight were measured using a correlation analysis. Values for fish length and fillet weight were log transformed to improve normality and meet the assumptions of parametric statistical analysis. A linear regression was then used to determine if fish weight, fish length and fillet weight explained variation in observed bruise weight. The differences in bruise areas between left and right fillets, and cut and skin sides of fillets were analyzed using a series of t-tests. A Rayleigh's test of uniformity was performed on bruise angles to determine whether bruises were uniformly distributed around the fillet centroid. The greater the test statistic (Z), the more closely the angles cluster around the mean vector, or the less uniform the points. Z is equal to nr^2, where n is the number of observations, and r is the length of the mean vector.

It was determined that 8.3 pixels were equal to 1mm. Fillet areas were calculated individually, and total fillet areas per fish were calculated by adding the areas of the inside of the left and right fillets. A significance level of 0.05 was used for all tests.

2.3 Results

2.3.1 Manual Assessment for Bruise Weight

Of the 156 fish sampled, 20 were male and 136 were female. Total fish length ranged from 325.0 – 505.0mm, with a mean of 399.0mm (s.d. = 29.6). Fish weights ranged from 290.0-1247.7g, with a mean of 524.9g (s.d. = 145.7). The fillets of the fish weighed between 90.0 and 393.3g, with a mean of 175.4g (s.d. = 49.3). The total fillet weight for

the entire batch was 27.36kg. The relationship between fish length and weight was defined by the equation $W = -4.63L^{2.82}$ ($r^2 = 0.71$), indicating that growth in yellowtail flounder is allometric. The average condition factor of the fish (K) was 0.81, ranging from 0.37 to 1.15. See Table 2.1 for descriptive statistics.

Females were significantly heavier and significantly longer than males ($t_{1,155} = 3.89$, $p < 0.01$; $t_{1,155} = 2.25$, $p = 0.02$). There was no gender-related difference in condition factor ($t_{1,155} = 1.75$, $p = 0.09$) or level of bruising ($t_{1,155} = 0.92$, $p = 0.36$).

Of all 156 fish sampled, 21 (13.5%) showed no bruising, while 135 (86.5%) showed at least some bruising. Sampled fish yielded between 0.0 and 32.1g of bruised tissue, with a mean of 4.5g (s.d = 6.1). The total bruise weight for the entire sample batch was 718.1g, a total yield loss of 2.6% by weight. Of the parameters measured, only fish weight was the only one found to be correlated with bruise weight ($r^2 = 0.15$, $p = 0.03$).

2.3.2 Digital Image Analysis for Bruise Area

Fillet areas ranged from 176.5cm^2 to 397.7cm^2 with a mean of 265.4cm^2 (s.d. = 43.4). Fillet area was well correlated with fish length, fish weight and fillet weight ($r^2 = 0.80$, $F_{1,307} = 0.90$, $p < 0.01$). Bruise area ranged from 0 to 124.5cm^2, with a mean of 12.1cm^2 (s.d. = 21.4), and accounted for an average of 4.3% of fillet area. The total bruise area for the entire sample batch was 3,453.3cm^2, which was 4.26% of the entire potential yield by area. The bruised areas of the insides of the fillets were equal to the bruise areas on the outsides of fillets in both left and right fillets ($t_{1,308} = 1.05$, $p = 0.30$; $t_{1,308} = 0.61$, $p =$

0.54). For both the left and right fillet, bruise areas on the outside of the fillets were not significantly different from those on the inside ($t_{1,311} = 0.07$, $p = 0.95$; $t_{1,305} = 0.19$, $p = 0.85$). Bruise area was not correlated with fish length, fish weight, fillet weight ($n = 156$, all $p > 0.05$), but it was correlated with bruise weight ($n = 156$, $r^2 = 0.30$, $p < 0.01$).

2.3.3 Bruise Locations

Bruises varied in their distance from the fillet centroid from 0.78mm to 142.68mm. They also varied in the circular distribution around the fillet centroid (see Table 2.2 for descriptive statistics for individual fillet information). According to the analysis, bruises were not uniformly distributed on any of the fillet sides (outside left: $Z_{1,272} = 49.21$, $p < 0.01$; inside left: $Z_{1,192} = 28.54$, $p < 0.01$; outside right: $Z_{1,374} = 39.91$, $p < 0.01$; inside right: $Z_{1,191} = 30.27$, $p < 0.01$). Angular histograms shown in Figure 2.8 reveal the strong tendency for bruising to occur at the anterior dorsal region of the fillet, commonly known as the "nape".

2.4 Discussion

2.4.1 Manual Assessment of Bruise Weight versus Digital Image Analysis of Bruise Area

Length and weight in yellowtail flounder was determined to be related to the equation $W = 4.63L^{2.82}$. The allometric coefficient, *b*, is in general agreement with previous studies conducted on flatfish, where it was found to range from 2.16-3.14 (Bayhan *et al,* 2006). Bruise weight was weakly correlated with fish weight and with no other factors. This

makes it difficult to recommend changes to processing/harvesting that will target fish with specific characteristics such as a minimum or maximum length or weight.

Fillet area was well correlated with fish length, fish weight and fillet weight. This indicates that the image analysis program was fairly accurate in its measurements of fillet areas, and in theory, bruise areas as well.

As expected, bruise area and bruise weight were found to be correlated, but not strongly. The total bruise area was 2.6% by weight, versus 4.3% by area. This could be due to a number of factors. First, it is possible that the digital image analysis is detecting discolouration that would not normally be removed upon manual assessment. Yellowing or browning of the flesh is at times acceptable to leave, depending on the severity and location. Removing slight, centrally located yellowing or browning often leads to a greater profit loss than just leaving it, as a slightly yellowed fillet may still have a greater value than a fillet that has been cut into numerous pieces.

Second, it is also possible that the image analysis program detected more bruising because the fillet photographs were taken on a light table, enhancing the appearance of bruising, whereas manual inspection took place on an opaque cutting board. The difference in yield loss by area and yield loss by weight may be minimized if manual inspection took place on a light table, or if the fillet photographs were taken on an opaque background.

2.4.2 Bruise Location

Results showed that bruises in the fillets of yellowtail flounder occur mainly in the anterior region of the fillet, commonly known as the "nape", and that it was consistent for both sides of the each fillet. To our knowledge, this is the first demonstrated case where fillet discoloration (bruising) has been shown to be non-random. Previously suggested explanations for the bruising in the nape included meshing (fish being caught in the trawl during capture) and physical damage due to rough handling during the gutting process. By visual comparison of bruised fillets with mesh lines on the skin of the whole fish, bruising due to meshing was ruled out as a possible cause of prominent bruising in the nape of the fillets. Evaluation of gutting technique also ruled out handling during processing as a direct cause of localized bruising in the nape.

One plausible explanation of prominent bruising in the nape may be the presence of the choroid-rete, a gas-regulating organ attached to the retina of the eye of some fish (including yellowtail flounder). In conjunction with pigment cell epithelium, the choroid rete maintains O_2 pressure in the eye and supplies blood to choriocapillaries (Barnett, 1951; Wittenburg and Wittenburg, 1974). Like the rete-mirable of the swim bladder, the choroid-rete is also a counter-current exchange system composed of thousands of closely arrayed capillaries (Barnett, 1951). Lactic acid released by the retinal pigment cell layer acidifies the blood in the choriocapillaries, which causes O_2 partial pressure in the eye to increase significantly. This increase is then multiplied by the counter current exchange in the rete mirable (Wittenburg and Wittenburg, 1974; Pelster and Weber 1991; Pelster and Randall, 1998). A rapid decrease in pressure on the gas within this organ, such as the one

associated with trawl ascent, can almost certainly be expected to rupture vessels, leading to internal hemorrhaging, or bruising. That said, bruising was not observed in all fish, indicating that there could be something else going on, such as varying external pressure effects due to varying position within the trawl.

2.5 Conclusion

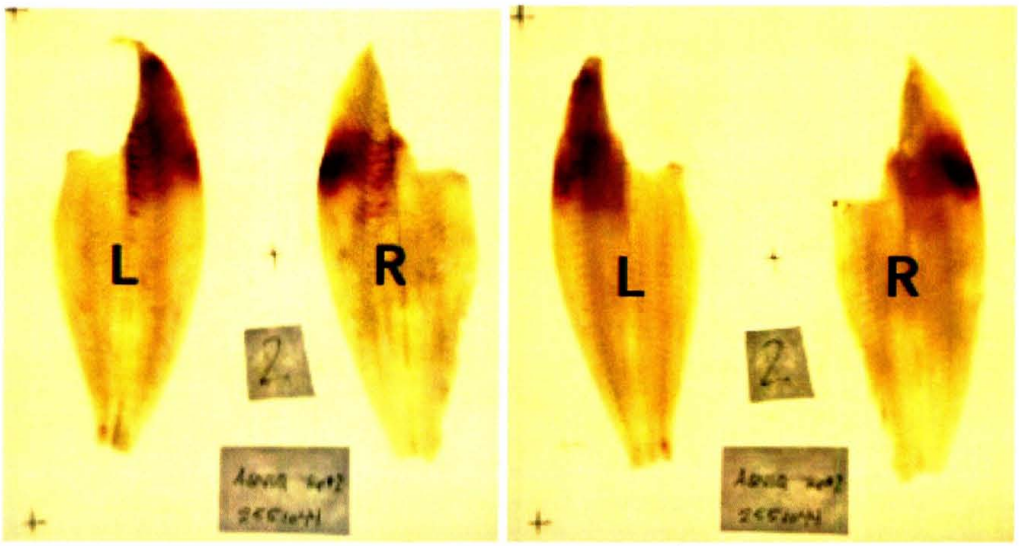
In conclusion, this study characterized the bruising patterns in commercially harvested yellowtail flounder, established a protocol for assessment of bruising, as well as a baseline level of bruising to which future levels of bruising can be compared. In order to better understand the prominence of bruising in the nape of the fillet, two recommendations are provided. First, an anatomical investigation of the vascular system of the yellowtail flounder is suggested to give a better idea of which vessels are associated with the majority of bruising, and thus potentially infer causation. Second, decompression trials are proposed in order to tease apart the effects of barotrauma from the impacts of being harvested by trawl. Simulated decompressions in a laboratory using a decompression chamber, as well as trials at sea are needed.

Table 2.1 Summary statistics for physical measurements taken from all fish during manual assessment.

	Minimum	Maximum	Mean	Standard Deviation
Fish Length (mm)	325	505	399	29.6
Fish Weight (g)	290	1247.7	524.9	145.7
Fillet Weight (g)	90	393.3	175.4	49.3
Fillet Area (mm^2)	17650.0	39770.0	26540.0	43.4
Condition Factor	0.37	1.15	.81	0.1
Bruise Area (mm^2)	0.0	12450.0	1210.0	21.4
Bruise Weight (g)	0.0	32.1	4.6	6.1

Table 2.2 Summary statistics for bruise information collected from each side of both fillets by digital photo analysis.

	Inside Left	Inside Right	Outside Left	Outside Right
Number of Bruises	193	192	273	375
Min. Bruise Angle (deg)	0.05	0	0.07	0.06
Max. Bruise Angle (deg)	359.92	359.91	360	359.91
Min. Bruise Distance from Centroid (mm)	7.31	2.04	5.75	0.78
Max. Bruise Distance from Centroid (mm)	142.28	127.03	142.68	137.00
Mean Vector (deg)	0.44	5.23	7.79	342.99
Length of Mean Vector (mm)	0.38	0.39	0.43	0.33
Median (deg)	2.32	358.95	359.80	357.81
Circular Variance	0.62	0.60	0.58	0.67
Circular Standard Deviation	79.28	77.88	75.00	85.76
Standard Error of Mean	7.30	7.06	5.50	6.24
Rayleigh's Test (Z)	28.54	30.27	49.21	39.91
Rayleigh's Test (p)	>0.01	>0.01	>0.01	>0.01



a b

Figure 2.1 Sample images of both the outside (a) and inside (b) of both the left (L) and right (R) fillets. Bruises are defined as regions of the fillet that are darker in colour than the surrounding fillet.

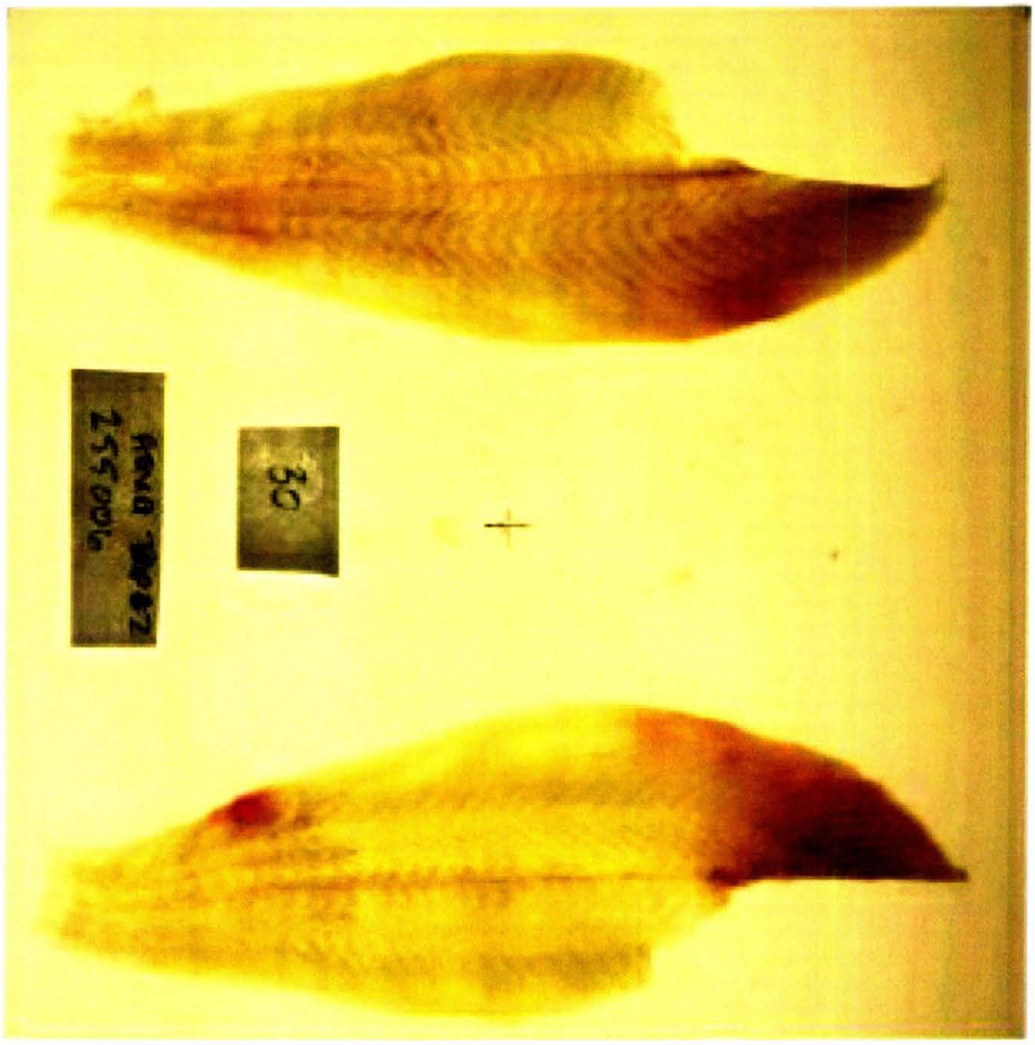


Figure 2.2 Sample input RGB (red, green and blue channels) image of the inside and outside of the left and right fillets.

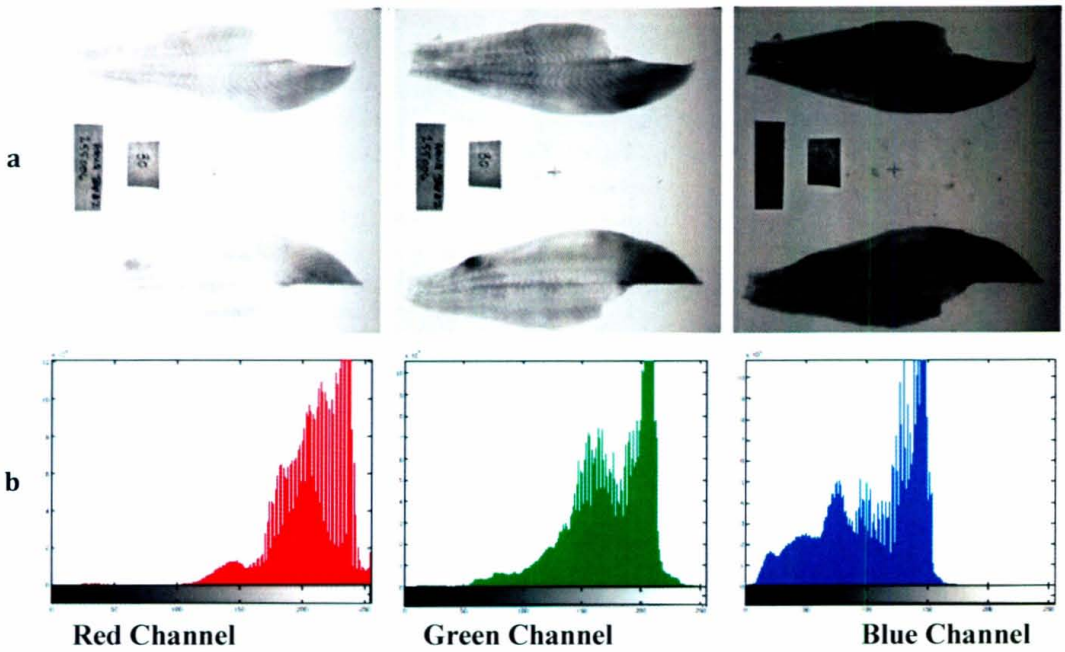
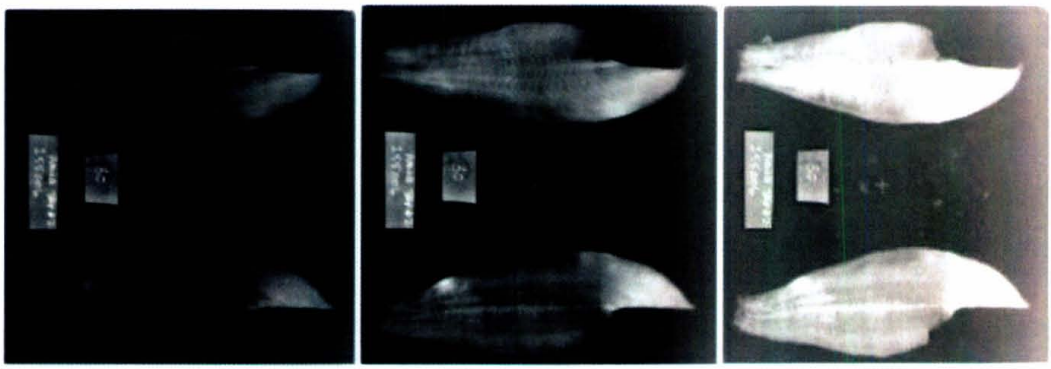


Figure 2.3 RGB colour channels (a) and the corresponding gray scale histograms (b) of Figure

Figure 2.4 Segmented image of RGB image in Figure 2.2, with fillet and background defined.



a b c

Figure 2.5 Inverted red (a), green (b) and blue (c) channels of RGB image in Figure 2.2. Regions of the fillet that are lighter in colour are defined as probable bruises. As can be seen from this image, the green channel was the best for bruise definition.

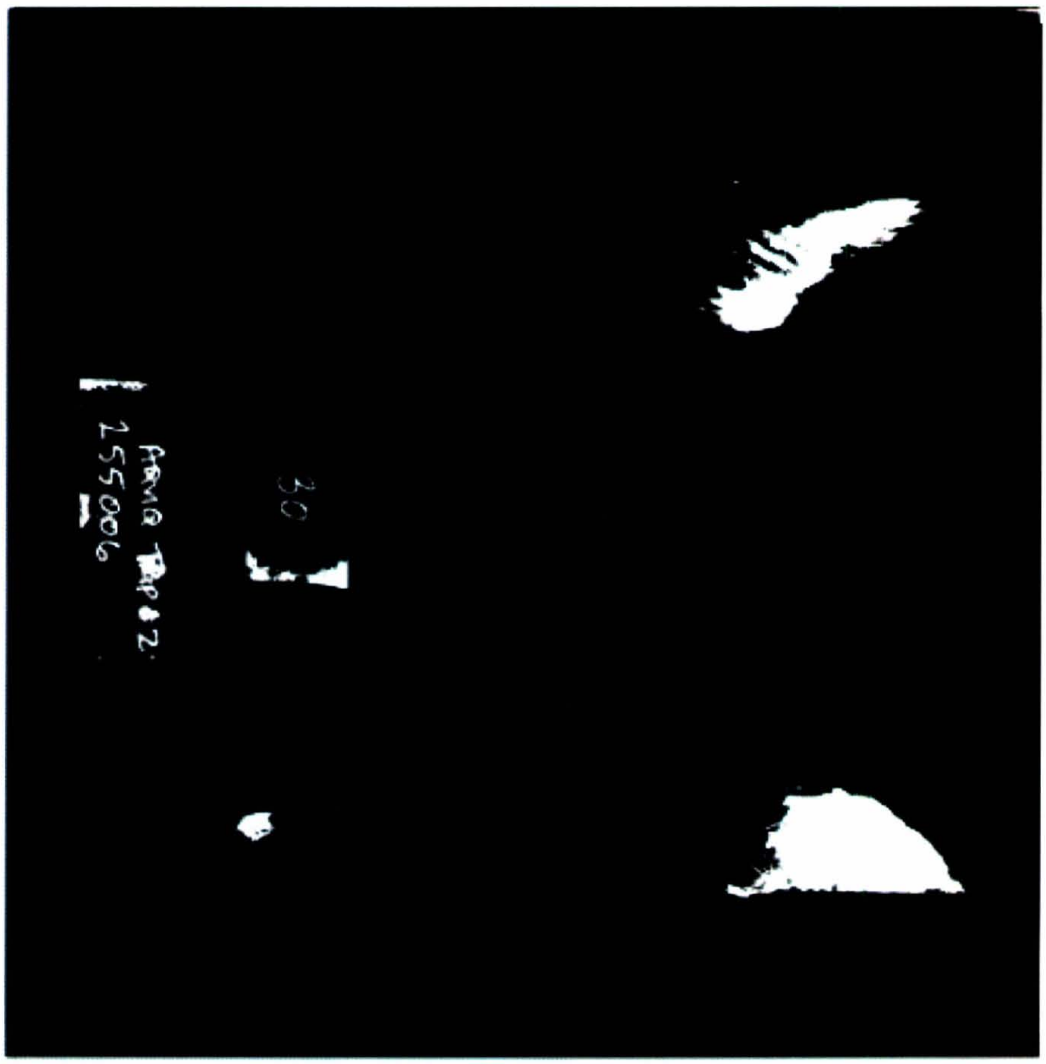


Figure 2.6 Segmented image of RGB image in Figure 2.2, with probable bruises defined in white.

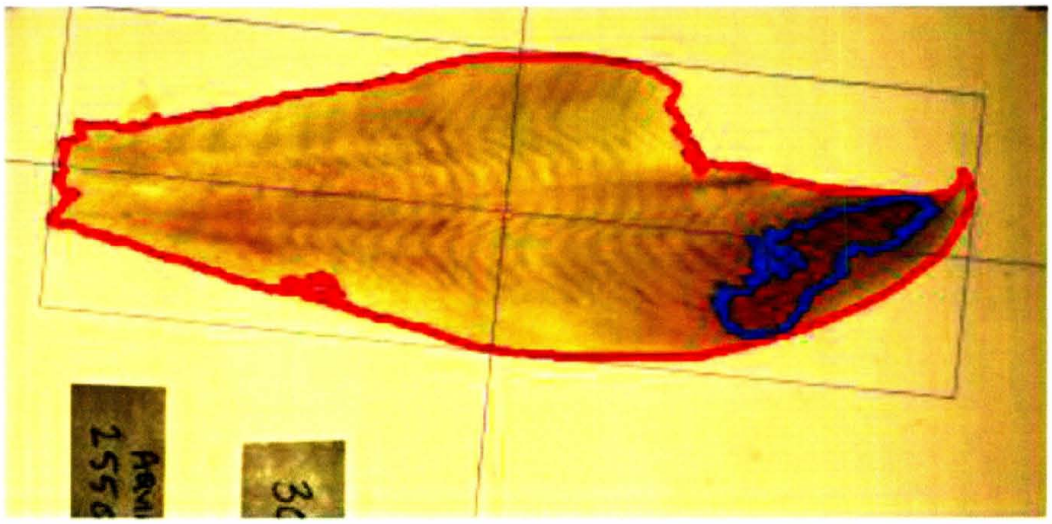


Figure 2.7 Geometric characterization of fillet and bruises. The red line represents the perimeter of the fillet, and the blue line is the perimeter of the area deemed bruised. The major (horizontal) and minor (vertical) axes of the fillet are shown. The intersection of the axes is the centroid, or geometrical centre of the fillet.

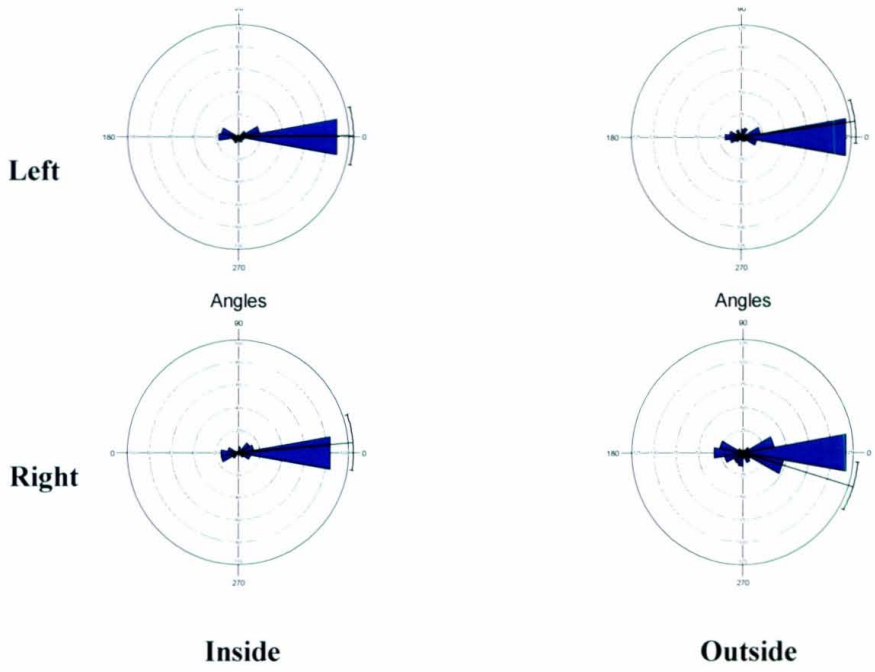


Figure 2.8 Angular histograms showing a non-uniform distribution of bruise angles relative to the fillet centroid of the left and right fillets, both inside and outside. Larger cones indicate greater bruise frequencies, and the solid line radiating from the centre indicates the mean vector.

Chapter 3. Factors affecting bruising in yellowtail flounder (*Limanda ferruginea).*

3.1 Introduction

Total global production for marine capture fisheries currently fluctuates between 80-90 million tonnes annually (FAO, 2010). Approximately 13,000 metric tons of flounder, three quarters of Atlantic Canada's landings, were harvested in Newfoundland alone in 2008, totaling nearly $8,000,000 of commercial revenue (Department of Fisheries and Oceans, 2008). In the case of whole fillet products, one of the most common challenges is fillet discolouration (bruising). There are a number of capture-related factors that contribute to fillet damage, including crowding in the net, meshing (fish becoming stuck in net mesh), and barotrauma. Once onboard the vessel, handling practices have also been shown to affect fillet discolouration (Ruff *et al.,* 2003), as does lipid oxidation of fresh fillets (Huss, 1995).Though discolouration, caused by physical or physiological trauma, has not been shown to affect the taste of fillets, it is disadvantageous for a number of reasons. Firstly, blood residue within the fillets has been shown to accelerate flesh softening (spoilage) post mortem due to lipid oxidation and hydrolysis of blood cells, which leads to proteolysis of the muscles and formation of hydrogen peroxide and hydroxyl radicals (Misra, 1972; Tretsven and Patten, 1981; Puppo and Halliwell, 1988a, 1988b; Ando *et al.,* 1999; Richards and Hultin, 2002; Grunwald and Richards 2006). Secondly, discolouration is visually unappealing to consumers, which negatively affects retail value (Tretsven and Patten, 1981; Connell, 1995; Huss, 1995; Robb *et al.*, 2003; Roth *et al.*, 2005; Olsen *et al.*, 2006). Because of this, it must be trimmed away prior to

packaging and distribution, which leads to a decrease in fillet weight, and consequently a loss in total yield and profit. For this reason, any practices that could lead to a decrease in fillet discolouration would be extremely valuable to the commercial fishing industry.

While approximately 80% of blood resides in the internal organs of a fish at rest, stressors or rapid actions, such as those associated with escape behaviours, gradually cause blood to be redirected from the organs to locomotory muscles (Thorarensen *et al.*, 1993). Stress has also been shown to reduce plasma clotting time (Fujika and Ikdea, 1985; Smit and Schoonbee, 1988; Ruis and Bayne, 1997), increase the production of thrombocytes and fibrin fibres crucial for clotting (Casillas and Smith, 1977), and increase blood viscosity (Gallagher *et al.*, 1995), making residual blood within muscle tissues more difficult to remove. It follows that immediate exsanguination, or bleeding, of slaughtered fish is extremely important (Kelly, 1969; Huss and Ansenjo, 1976; Valimarsson *et al.*, 1984; Botta *et al.*,1986; Warris and Wilkins, 1987; Roth *et al.*, 2005).

It is widely accepted that adequate bleeding is necessary in large commercial fish for a high quality product (Kelly and Little, 1966; Roth *et al.*, 2007). It has been shown that proper bleeding reduces blood spots in smoked salmon (Michie, 2001). There is, however, disagreement as to which bleeding method is the most effective (Huss, 1995). Currently, commercially harvested yellowtail flounder are slaughtered via evisceration (or gutting), which entails manual removal of viscera prior to bleeding and freezing. It has been suggested that a beating heart is critical to effective bleeding, but numerous studies have shown this is not necessarily the case (Huss and Ansenjo, 1976; Warris and

Wilkins, 1987; Robb *et al.*, 2003; Roth *et al.*, 2005). Alternative methods of slaughter include slicing two or more of the gill arches (gill slit), or removal of the tail (bobtail) prior to bleeding. Previous research has demonstrated the increased effectiveness of evisceration over gill slitting (Olsen *et al.*, 2006), which is intuitive, as only blood present in and near the gills is purged when gills are cut (Hoar and Randall, 1970), but limited research has been conducted on the effectiveness of the bobtail method. Optimal time spent bleeding out post-slaughter has also been debated, with recommendations ranging from 12 to 60 minutes in cold sea water, or head down in air for 0-24 minutes (Olsen *et al.*, 2006; Roth *et al.*, 2009). Regardless of the duration of the bleed, the first five minutes have been shown to be the most crucial to bleed effectiveness (Roth *et al.*, 2005), and cold water is optimal for the bleed tank, as chilling delays blood coagulation (Connell, 1995).

The purpose of this study was to investigate the impact of three slaughter methods (gutting, bobtail and gill slit), bleed time (5, 10 and 20 minutes), and freezer orientation (vertical and horizontal) on the total area (mm^2) of discolouration (hereafter called bruising), a result of residual blood remaining in fillets. Based on pervious research it is predicted that bruising will be less in fish slaughtered by evisceration than those by gill slitting. It is hypothesized that bruising will decrease with increased time spent in the bleed tank. It is also hypothesized that bruising will be less in fish frozen in vertical plate freezers due to the increased ease with which residual blood may drain from the body. Any knowledge gained as a result of this study will be used to further increase the efficiency of the commercial yellowtail flounder industry.

3.2 Methods and Materials

3.2.1 Experiment I

In February 2010, a series of nine treatments were carried out aboard the OCI operated flatfish trawler *FV Aqviq* to evaluate the effects of three different bleeding methods (gutting, gill slitting and bobtailing), and three different durations of time spent in the bleed tank (5, 10 and 20 minutes) on overall fillet bruising (Table 3.1). For the purpose of this experiment, the gutting treatment was used as the control, as it is the current standard technique. Fish were removed from the picking belt of the conveyor as the fish exited the holding ramp. Each fish was individually tagged (Floy tag) and assigned to a treatment group, and measured for total length.

Fish included in the gutting treatment had their viscera removed quickly with a filleting knife. Fish in the gill slitting treatment had their gill arches cut, but internal organs remained intact. Fish in the bobtailing treatment had their tails removed just anterior of the caudal peduncle, and internal organs remained intact.

Each experimental fish was measured for length and weight prior to processing. Bruises on the blind side and then the eyed side of the whole animal were identified numerically (1, 2, 3, 4, etc) and their approximate location and size/shape (mm^2) documented on the fillet image (legend) included on each data sheet. A data sheet was dedicated to each fish, and each data sheet had an image (legend) of the fillet for both the blind and eyed

side of the fish. Blood pooling occurring on the fringe (within fins and fin ray attachments to the body) were not recorded, as this is removed during filleting. The numerical reference to each bruise was recorded on the data sheet in the space below the fillet image. Below each fillet image there were two columns, one labeled 'before' (bleed method × bleed time treatment is carried-out) and one labeled 'after' (bleed method × bleed time treatment is carried-out). Each bruise was measured in a linear fashion in which the longest length and longest width were recorded before treatment, and again after treatment.

Once all fish had been assessed for level of bruising, they were placed in a fine-mesh bag and placed in the bleed tank for the duration of the bleed time treatment being performed. Upon completion of the assigned bleed time, the fish were removed and re-assessed as above.

The above process was repeated until approximately 45 (+/-6) fish were processed for each of the 9 treatment groups, totaling 414 fish (Table 3.1). Frozen fish were shipped to the Fisheries and Marine Institute of Memorial University in St. John's, NL, where each animal was identified by its tag number, filleted by hand, and then the fillets assessed for level of bruising. Bruises that had been measured externally and were not present internally were recorded as zero.

3.2.2 Experiment II

Experiment II was conducted in November 2010 aboard the same vessel. Based on the initial bleed method/bleed time trials in Experiment I, the experimental design in the second experiment was modified to remove the gill slit method. Consultation with the industry partner revealed that this technique was not practical, as it was shown to be more complex, time-consuming and to require additional training for the crew. Otherwise, the procedure for the repeat trials was the same as for the initial trials.

In addition, two treatment groups were included to evaluate the effect of plate freezer orientation on fillet bruise area: vertical and horizontal freezer orientation. For each experimental treatment (i.e., vertical or horizontal), a random sample of 170 yellowtail flounder exhibiting some level of bruising were removed from the picking belt of the conveyor as they exited the holding ramp of the vessel and were assigned to one treatment group or the other. Note: fish selected had to possess some level of bruising as the experiments were designed to test the effect of the bleed method and bleed time on fillet discolouration. Following treatment group assignment, individual fish were gutted and bled according to OCI company protocol, and were then flash frozen according to treatment group, and stored below zero degrees Celcius for shipment to the Fisheries and Marine Institute for bruise assessment.

3.2.3 Analysis

In Experiment I, total internal bruise area in mm^2 was calculated as the sum of the areas of all of the bruises measured on an individual. The effects of fish length and fish weight on total internal bruise area were analyzed using regression analysis. A t-test was used to investigate the effect of sex on total internal bruise area. All externally visible bruises were measured individually before and after bleed (greatest length by greatest width of bruise), and compared to the internal bruises using regression analysis. The effects of bleed method and bleed time on total internal bruise area were first evaluated using a two-way ANOVA.

In Experiment II, the effects of fish length, fish weight, sex, external bruise area, bleed method and bleed time on total internal bruise area were analyzed similar to Experiment I. The effect of freezer orientation on internal bruise area was tested using a one-way ANOVA. Internal bruising was not significantly different between males and females, and therefore sexes were grouped together for analysis.

3.3 Results

3.3.1 Experiment I

A total of 414 fish were assessed, 293 of which were female, and 137 of which were male. The mean fish length was 38.2cm (SD = 3.50), with a range of 31 to 48cm. The mean fish weight was 421g (SD = 134.0), with a range of 190 to 1030g. See Table 3.1 for

descriptive statistics. Females were significantly heavier and longer than males ($t_{1,413}$ = 9.88, $p < 0.01$; $t_{1,413} = 9.52$, $p < 0.01$).

Of the 414 fish sampled, 384 individuals showed at least some degree of internal bruising (92.8%). Total internal bruise area varied between 0 and 12800 mm^2 (mean = 248.20, SD = 626.40). Fish length did not explain a significant portion of the variation in internal bruise area observed ($F_{1,413} = 3.90$, $p = 0.05$, $r^2 = 0.01$; Figure 3.1), and while fish weight was significant, it explained only 3% of variation in internal bruise area ($F_{1,413} = 14.29$, $p < 0.01$, $r^2 = 0.03$; Figure 3.2). A comparison of the mean internal bruise area for each sex revealed no difference in internal bruise area between males and females ($t_{1,413} = -1.38$, $p = 0.17$; Figure 3.3).

External bruise area before bleed varied between 0 and 10500 mm^2 (mean = 455.96, SD = 867.89). Once bled, external bruise area for the same individuals varied between 0 and 10500 mm^2 (mean = 461.61, SD = 922.14). Presented graphically, Figure 3.4 reveals that the external bruise areas measured before bleed are a good predictor of external bruising after bleed ($F_{1,1116} = 3635.14$, $p = <0.01$, $r^2 = 0.76$). However, the results showed that the external bruise area measured before and after bleed only explained 17% and 21% respectively of the variation in internal bruise area ($p < 0.05$; Figures 3.5 and 3.6). These findings suggest that external bruise area is not a good predictor of internal bruise area (see further comment in the Discussion).

Results from the two-way analysis of variance revealed that bleed method significantly affected internal bruise area ($F_{8,413} = 4.27$, $p = 0.01$; Figure 3.7). Post hoc comparisons between the different treatments showed that the mean internal bruise area in bobtailing and gill slitting were not significantly different from the control treatment, gutting ($p > 0.05$). However, the mean internal bruise area for individuals in the bobtail treatment was significantly lower than the gill slit treatment ($p < 0.05$).

Internal bruise area did not significantly vary across the different bleed time treatments ($F_{8,313} = 0.14$, $p = 0.87$; Figure 3.8). Neither was there any significant interaction between the factors, bleed method and bleed time ($F_{8, 313} = 0.67$, $p = 0.62$). See Figure 3.9 for boxplots, and Table 3.2 for descriptive statistics.

3.3.2 Experiment II

A total of 314 fish were assessed, 248 of which were female, and 66 of which were male. The mean fish length was 38.5cm (SD = 3.3), with a range of 31 to 51cm. The mean fish weight was 491.92g (SD = 158.87), with a range of 218 to 1185g. Females were shown to be significantly heavier and longer than males ($t_{1,313} = 7.83$, $p < 0.01$; $t_{1,313} = 7.35$, $p < 0.01$). The mean fish length found in Experiment II was not significantly different than the mean fish length in Experiment I ($t_{1, 727} = 1.11$, $p = 0.27$; Figure 3.10), however fish were significantly heavier in Experiment II than those observed in Experiment I ($t_{1,727} = 6.52$, $p = <0.01$; Figure 3.11).

Of the 314 fish sampled, 299 individuals showed some amount of internal bruising (95.2%), up slightly from Experiment I. The areas of individual internal bruises varied between 0 and 5,400mm^2 with a mean of 379.97 (SD = 652.41). Total internal bruise area per fish varied between 0 and 5,400mm^2 with a mean of 659.81 (SD = 921.35). This was not significantly different than the mean bruise area documented in Experiment I ($t_{1,742}$ = 0.19, p = 0.85; Figure 3.12). See Table 3.2 for descriptive statistics.

Fish length and fish weight were not significantly correlated with internal bruise weight, both explaining less than 1% of observed variartion ($F_{1,312}$ = 1.09, p = 0.29, r^2 < 0.01; $F_{1,312}$ = 1.65, p = 0.19, r^2 < 0.01; Figure 3.13 and Figure 3.14). A comparison of the means for each sex revealed no significant difference between males and females ($F_{1,312}$ = 1.79, p = 0.18; Figure 3.15).

External bruise area before bleed varied between 0 and 5700 mm^2 (mean = 556.64, SD = 756.17). Once bled, external bruise area for the same individuals varied between 0 and 4650 mm^2 (mean = 581.77, SD = 671.09). Presented graphically, Figure 3.16 reveals that the external bruise areas measured before bleed are a good predictor of external bruising after bleed ($F_{1,548}$ = 737.45, p = <0.01, r^2 = 0.57). The results also revealed that the external bruise area measured before bleed explained 11% of the variation in internal bruise area ($F_{1,548}$ = 70.06, p = <0.01, r^2 = 0.11; Figure 3.17) and that external bruise area measured after bleed explained 23% of the variation in internal bruise area ($F_{1,548}$ = 163.79, p = <0.01, r^2 = 0.23; Figure 3.18).

Internal bruise area was not statistically different between the different bleed methods ($F_{3,313}$ = 1.45, p = 0.23) or bleed times ($F_{3,313}$ = 0.30, p = 0.58). Neither was there significant interaction between the factors ($F_{3,313}$ = 0.07, p = 0.78).

Of 170 fish assessed in the freezer orientation trials, 144 had some degree of internal bruising (84.7%). Of fish frozen in the vertical plate freezer, 87.7% were bruised, significantly more than those frozen in the horizontal plate freezers, which were 81.25% bruised ($F_{1,168}$ = 7.32, p < 0.01; Figure 3.22). Total internal bruising for fish frozen in vertical plate freezers ranged from 0 to 8,850 mm^2, with a mean of 1,440.42 mm^2 (SD = 2049.56). Total internal bruising for fish frozen in horizontal plate freezers ranged from 0 to 68,000 mm^2, with a mean of 774.15 mm^2 (SD = 1254.56). See Table 3.3 for descriptive statistics.

3.4 Discussion

3.4.1 Experiment I

In the February trials, weight was a significant predictor of bruising in yellowtail flounder, though length was not significant. This finding agrees with previous data (see Chapter 2). There was no significant difference in bruising between males and females. Consistent with expected results, external bruise area measured after bleeding increased linearly with external bruise area measured before bleeding, explaining 76% of the variation. External bruise area before bleed predicted only 17% of the variation in internal bruise area, and external bruise area after bleed predicted 21% of variation.

Though both relationships were significant, the correlations were not strong enough to recommend external bruise area be used as an indicator of the internal condition of the fish unless the external bruising is obviously severe.

Internal bruise area in fish in the bobtail and gill slit treatments was not significantly different than bruise area in the gutting (control) treatment. This is contrary to previous research on salmon that demonstrated an increased effectiveness of gutting over gill slitting (Olsen *et al.*, 2006). Gill slitting is still somewhat effective because it targets the gills, which are a highly vascularized part of the fish, but still only blood present in and near the gills is purged when gills are cut (Hoar and Randall, 1970).

The amount of time spent in the bleed tank had no significant effect on bruise area. It was hypothesized that internal bruise area would decrease with time spent in the bleed tank, as there would be more time for exsanguination to occur. Fish in the gill slit treatment did show the expected decrease in mean bruise area with increasing time in the bleed tank (albeit not significant), but still displayed the highest overall levels of bruising. Gutted fish had slightly less bruising with increased time spent in the bleed tank. While this was not significant, bruising was still approximately 10% less after 20 minutes in the bleed tank than after five minutes, which follows the expected trend. In the bobtailed fish, bruising actually increased with time spent in the bleed tank (albeit not significantly), which is the opposite of what one might expect. A functional explanation for this finding is currently lacking, but it might be a result of a weakness in the experimental design. For

this reason, bobtailing and gutting were identified as potential effective bleed methods, and were repeated in Experiment II.

3.4.2 Experiment II

In Experiment II, fish were not significantly different in length compared to Experiment I, but they were significantly heavier. This weight discrepancy may be due to the fact that Experiment I was undertaken in February and Experiment II was undertaken in November. This seasonal difference is relevant because yellowtail flounder spawn in the spring and summer, peaking in June (Pitt, 1970), and so can be expected to be in peak physical condition in the late fall when muscle mass is fully regained after spawning, and has yet to be depleted as energy is diverted from somatic growth to reproduction. Another potential reason that yellowtail were heavier in November than in February may have been the fact that food is typically less available during the winter months. A significant portion of the yellowtail diet is composed of the northern sand lance (*Ammodytes dubius*) (Bruno *et al.* 2000). It inhabits the northern Atlantic and Pacific oceans. Sand lance spawning occurs between the months of November and February and entails open ocean populations migrating inshore to spawn in shallow waters and on sandy beaches (Hart, 1973). This would make sand lance populations less accessible to yellowtail during the winter months, and thus potentially reduce a significant portion of their diet. Yellowtail may be heavier in November because by February they have had limited access to sand lance for the winter months. This is most applicable for larger yellowtail, as sand lance account for a larger part of the diet.

Internal bruise area was not significantly different between Experiment I and II. As in Experiment I, internal bruise area was not predicted by fish length. Contrary to Experiment I, internal bruise area in Experiment II was not significantly correlated with fish weight. This could possibly be attributed to the difference in weights between the experiments.

Similar to results in Experiment I, external bruise area measured after bleeding increased linearly with external bruise area measured before bleeding, explaining 57% of the variation. External bruise area before bleed predicted only 11% of the observed variation in internal bruise area, and external bruise area after bleed predicted 23% of variation. These results were consistent with Experiment I, and support the conclusion that external bruise area can somewhat predict internal bruise area, but should not be used as the sole indicator of internal condition.

Bleed method (gutting versus bobtail) had no significant effect on the resulting internal bruise area. This is consistent with the results from Experiment I. Recognizing that the majority of a fish's blood resides within the gut, and that only 20% is typically found in the muscular tissues (Thorarensen *et al.*, 1993), it was expected that the removal of the gut would be more effective than bobtailing at reducing fillet discoloration if blood is mobilized from this region. While both experiments suggest gutting to be no more effective than bobtailing, functional explanations for this finding remain unclear, and support the need for further investigation, particularly temporal or seasonal variation throughout the year.

Similar to Experiment I, the amount of time spent in the bleed tank had no significant effect on the resulting internal bruise area. In the gutting treatment however, there was a decrease (albeit insignificant) in bruise area between 5 and 10 minutes spent in the bleed tank. Inexplicably, bruising increased after the 10 minute treatment. This contradicted results in Experiment I, so it is recommended that for the time being, fish should spend at least 5-10 minutes in the bleed tank for effective bleeding, and that more research be conducted on the effects of bleed time.

Fish frozen in the vertical plate freezers had a significantly higher level of bruising than those frozen in horizontal plate freezers. One possible reason that this may have been the case is that by virtue of their orientation, vertical freezers are easier to load carelessly. Over-loading the freezers may increase bruising of the fillets when the plates are closed. FAO suggests that fish not be over crowded in a vertical plate freezer, and specifically not be loaded above the blocks (FAO, 1970). Another potential explanation for this observation is that while there is no specific company procedures, crew members may selectively load horizontal freezers with fish that appear to be less bruised due to the fact that the end product from the horizontal freezers are whole, gut in fish. Consumers of this product would be more concerned with the external appearance of fish than consumers of fillets or other factory processed products.

3.5 Conclusion

In conclusion, this study investigated the impact of bleed method, bleed time and freezer orientation on total area of residual blood remaining in the fillets after processing, commonly known as bruising. It is recommended that the industry continue to use gutting as an effective method of bleeding, and that gutted fish should be held between 5 and 10 minutes in the bleed tank to ensure effective exsanguination. Fish should be frozen in a horizontal plate freezer, or more care should be used when loading the vertical plate freezer so as not to cause unnecessary fillet damage. External bruise area is an indicator of internal bruise area but should not be used as the sole indicator of internal condition. It is recommended that further research be carried out on bleed time, as between the two experiments, no conclusion was reached.

Table 3.1 Descriptive statistics for fish assessed in Experiment I, broken down by bleed method/bleed time treatments.

		Minimum	Maximum	Mean	St. Dev.
Gut 5	*Length (mm)*	310	530	395.8	35.2
	Weight (g)	190	1340	395.8	173.8
	*EBABB (mm^2)**	0	10500	1003.3	1558.6
	*EBAAB (mm^2)***	0	10500	1132.2	1634.4
	Ind. IBA (mm^2)$^+$	0	12800	297.2	1137.7
	Tot. IBA (mm^2)$^{++}$	0	5725	797.66	1299.2
Gut 10	*Length (mm)*	320	480	381.0	31.6
	Weight (g)	240	820	406.3	113.4
	EBABB (mm^2)	0	9950	1188.5	1723.5
	EBAAB (mm^2)	0	9645	1084.5	1660.3
	Ind. IBA (mm^2)	0	5600	237.8	569.3
	Tot. IBA (mm^2)	0	4450	619.9	989.4
Gut 20	*Length (mm)*	330	440	373.2	30.0
	Weight (g)	240	680	384.3	111.7
	EBABB (mm^2)	0	5197	960.2	1178.3
	EBAAB (mm^2)	0	6231	924.9	1207.6
	Ind. IBA (mm^2)	0	5600	216.0	550.6
	Tot. IBA (mm^2)	0	6250	608.7	1035.6
Bobtail 5	*Length (mm)*	320	440	374.6	34.3
	Weight (g)	240	720	407.3	122.6
	EBABB (mm^2)	0	3860	1104.8	1045.8
	EBAAB (mm^2)	0	4780	1146.2	1089.5
	Ind. IBA (mm^2)	0	1050	150.9	214.4
	Tot. IBA (mm^2)	0	1800	390.4	444.8
Bobtail 10	*Length (mm)*	330	480	383.54	28.7
	Weight (g)	240	930	420.2	124.8
	EBABB (mm^2)	0	5444	1027.2	1174.8
	EBAAB (mm^2)	0	5375	5375	1071.2
	Ind. IBA (mm^2)	0	1650	190.0	321.9
	Tot. IBA (mm^2)	0	2100	407.8	551.0
Bobtail 20	*Length (mm)*	340	480	391.1	30.03
	Weight (g)	340	1020	472.9	127.4
	EBABB (mm^2)	0	6298	821.9	1097.1
	EBAAB (mm^2)	0	6123	830.1	1045.2
	Ind. IBA (mm^2)	0	6400	253.9	702.2
	Tot. IBA (mm^2)	0	6400	630.92	1087.6

Gill Slit 5	*Length (mm)*	320	680	389.2	54.9
	Weight (g)	240	1030	435.3	150.2
	EBABB (mm^2)	0	7960	1402.5	1646.9
	EBAAB (mm^2)	0	9450	1354.0	1800.2
	Ind. IBA (mm^2)	0	5500	299.1	669.5
	Tot. IBA (mm^2)	0	3675	593.9	821.8
Gill Slit 10	*Length (mm)*	330	440	383.8	26.6
	Weight (g)	250	720	424.1	104.5
	EBABB (mm^2)	0	10480	1683.5	1869.8
	EBAAB (mm^2)	0	11820	1637.7	2075.3
	Ind. IBA (mm^2)	0	3000	325.6	549.4
	Tot. IBA (mm^2)	0	4600	911.7	1133.1
Gill Slit 20	*Length (mm)*	320	470	390.6	35.6
	Weight (g)	220	980	461.3	150.0
	EBABB (mm^2)	0	6227	1228	1148.5
	EBAAB (mm^2)	0	5748	1373.9	1221.8
	Ind. IBA (mm^2)	0	2100	234.4	382.3
	Tot. IBA (mm^2)	0	3600	701.6	775.5

*EBABB = External area of an individual bruise before bleed
**EBAAB = External area of an individual bruise after bleed
$^{+}$Ind. IBA = Internal area of an individual bruise
$^{++}$Tot. IBA = Total of all internal bruise areas of one individual animal

Table 3.2 Descriptive statistics for fish assessed in Experiment II, broken down by bleed method/bleed time trials.

		Minimum	Maximum	Mean	St. Dev.
Gut 5	*Length (mm)*	31	47	38.3	3.1
	Weight (g)	240.8	1184.6	477.1	159.7
	EBABB (mm^2)	0	2720	506.2	561.6
	EBAAB (mm^2)	0	3045	558.0	641.7
	Ind. IBA (mm^2)	0	3750	396.2	676.2
	Tot. IBA (mm^2)	0	1184.6	702.9	1024.1
Gut 10	*Length (mm)*	32	51	39	3.5
	Weight (g)	269.1	1176.4	503.9	173.8
	EBABB (mm^2)	0	4816	532.6	736.9
	EBAAB (mm^2)	0	2295	495.6	486.6
	Ind. IBA (mm^2)	0	1265	264.5	315.6
	Tot. IBA (mm^2)	0	2490	428.0	540.3
Gut 20	*Length (mm)*	32	46	38.4	2.9
	Weight (g)	256.6	932.2	470.3	132.4
	EBABB (mm^2)	0	2850	520.3	623.4
	EBAAB (mm^2)	0	2944	586.5	610.7
	Ind. IBA (mm^2)	0	4675	384.2	685.1
	Tot. IBA (mm^2)	0	4825	682.2	895.0
Bobtail 5	*Length (mm)*	330	450	382.4	27.7
	Weight (g)	285.4	844.1	462.2	135.1
	EBABB (mm^2)	0	5700	613.1	859.2
	EBAAB (mm^2)	0	3502	672.8	711.3
	Ind. IBA (mm^2)	0	4500	376.7	633.5
	Tot. IBA (mm^2)	0	4500	690.6	877.3
Bobtail 10	*Length (mm)*	310	480	382.4	35.8
	Weight (g)	218.5	990	493.5	161.0
	EBABB (mm^2)	0	5700	584.4	884.7
	EBAAB (mm^2)	0	4365	627.2	761.8
	Ind. IBA (mm^2)	0	3600	419.2	688.2
	Tot. IBA (mm^2)	0	5146	766.6	1076.0

Bobtail 20	*Length (mm)*	340	510	391.7	37.4
	Weight (g)	303.2	978.4	545.8	173.3
	EBABB (mm^2)	0	5625	565.3	880.1
	EBAAB (mm^2)	0	4650	563.2	777.0
	Ind. IBA (mm^2)	0	5400	431.1	803.8
	Tot. IBA (mm^2)	0	5400	790.3	1121.0

*EBABB = External area of an individual bruise before bleed
**EBAAB = External area of an individual bruise after bleed
[+]Ind. IBA = Internal area of an individual bruise
[++]Tot. IBA = Total of all internal bruise areas of one individual animal

Table 3.3 Bruise statistics for fish assessed in freezer orientation trials in Experiment II.

		Minimum	Maximum	Mean	Standard Deviation
Vertical	Left Fillet Internal Bruise Area (mm^2)	0	243.75	3500	562.20
	Right Fillet Internal Bruise Area (mm^2)	0	396.81	7700	1134.61
	Total Bruise Area (mm^2)	0	1440.42	8850	2049.56
Horizontal	Left Fillet Internal Bruise Area (mm^2)	0	181.82	4125	479.54
	Dark Fillet Internal Bruise Area (mm^2)	0	170.71	3600	415.84
	Total Internal Bruise Area (mm^2)	0	774.15	6800	1254.56

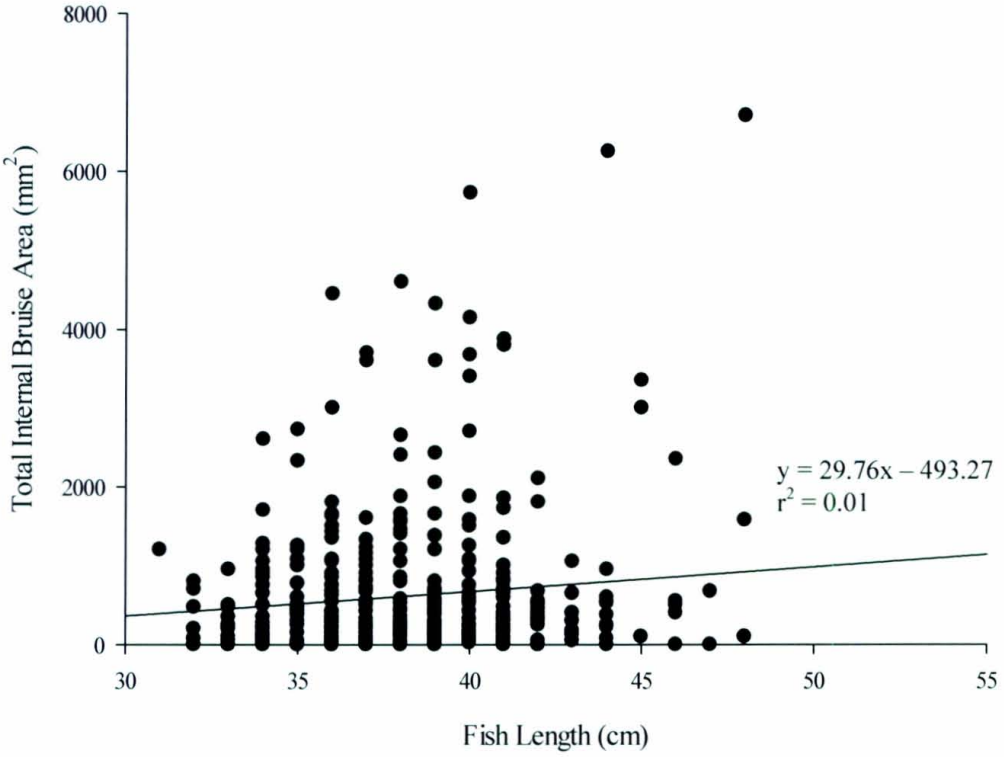


Figure 3.1 Total internal bruise area (mm^2), calculated as the sum of the areas of all the bruises measured on a given fish, plotted by fish length (cm). Internal Bruise Area = 29.77*Fish Length – 493.27, $r^2 = 0.01$.

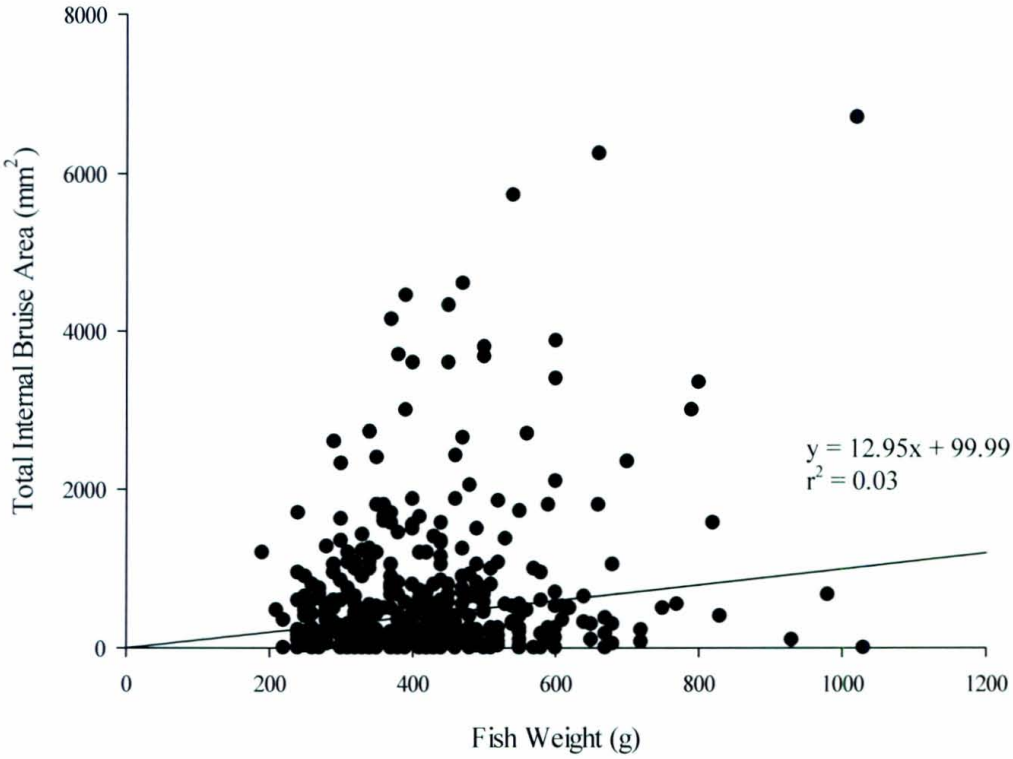


Figure 3.2 Total internal bruise area (mm^2), calculated as the sum of the areas of all the bruises measured on a given fish, plotted by fish weight (g). Internal Bruise Area = 12.95*Fish Weight + 99.99, $r^2 = 0.03$.

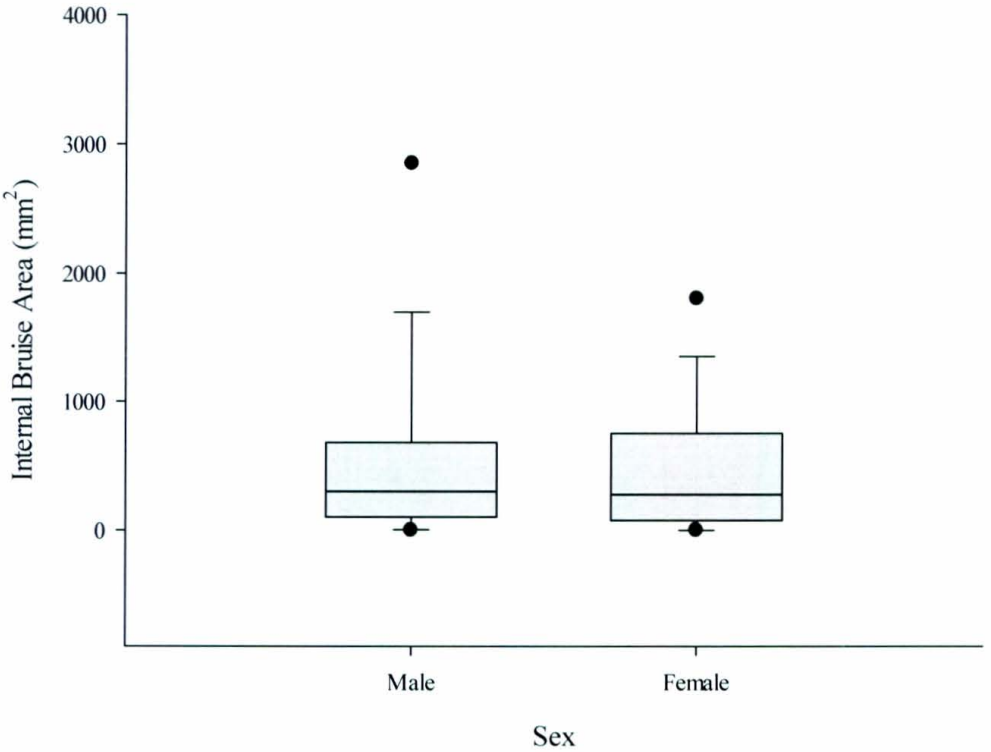


Figure 3.3 Mean internal bruise area (mm^2), calculated as the mean of the areas of all the bruises measured on a given fish, plotted by sex.

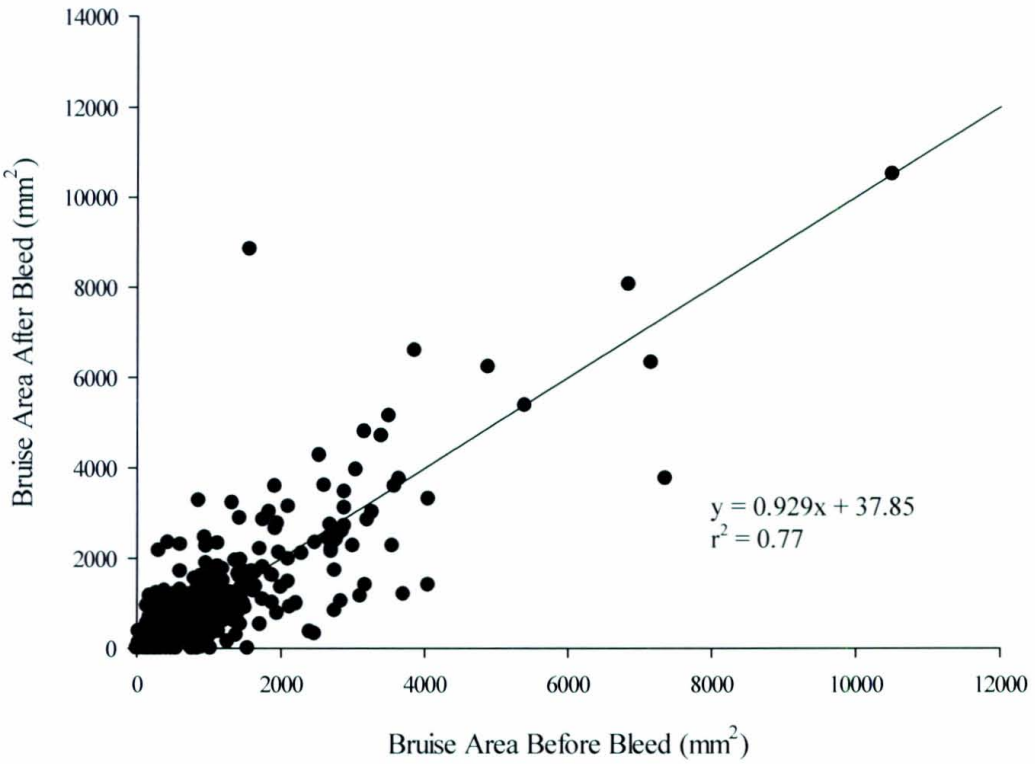


Figure 3.4 External area of a single bruise after bleed plotted by the external area of that same bruise before bleed (mm^2). External Bruise Area After Bleed = 0.929*External Bruise Area Before Bleed + 37.85, $r^2 = 0.77$.

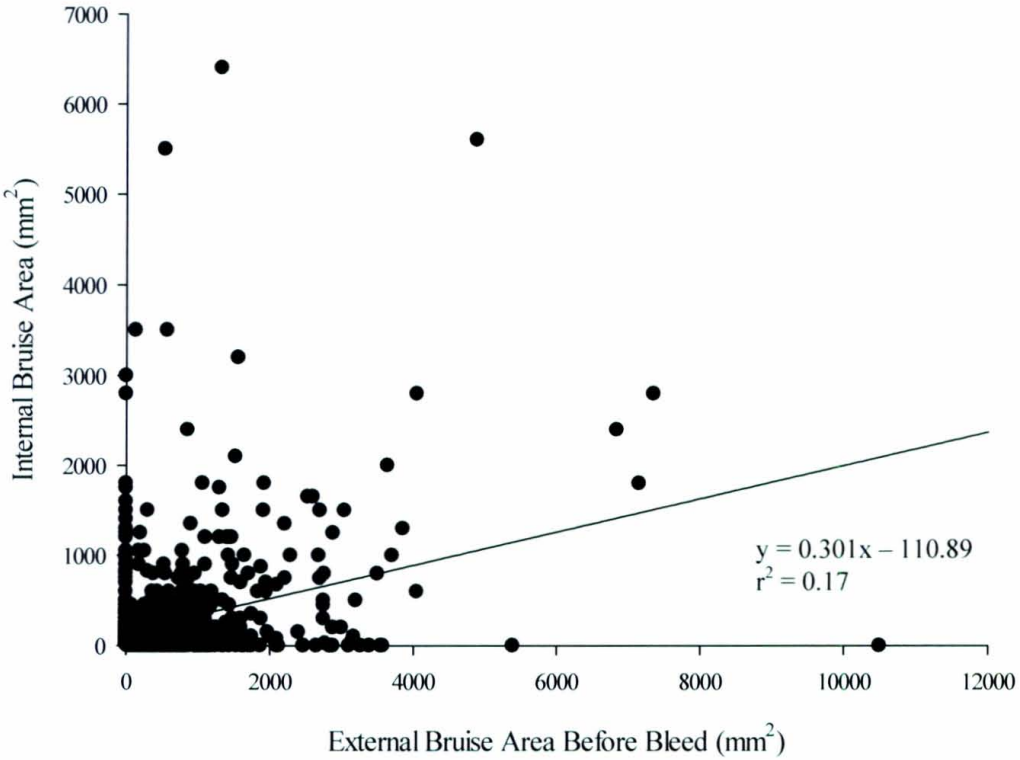


Figure 3.5 Internal area of a single bruise (mm^2) plotted by the external area of that same bruise before bleed (mm^2). Internal Bruise Area = 0.301*Bruise Area Before Bleed + 110.89, $r^2 = 0.17$.

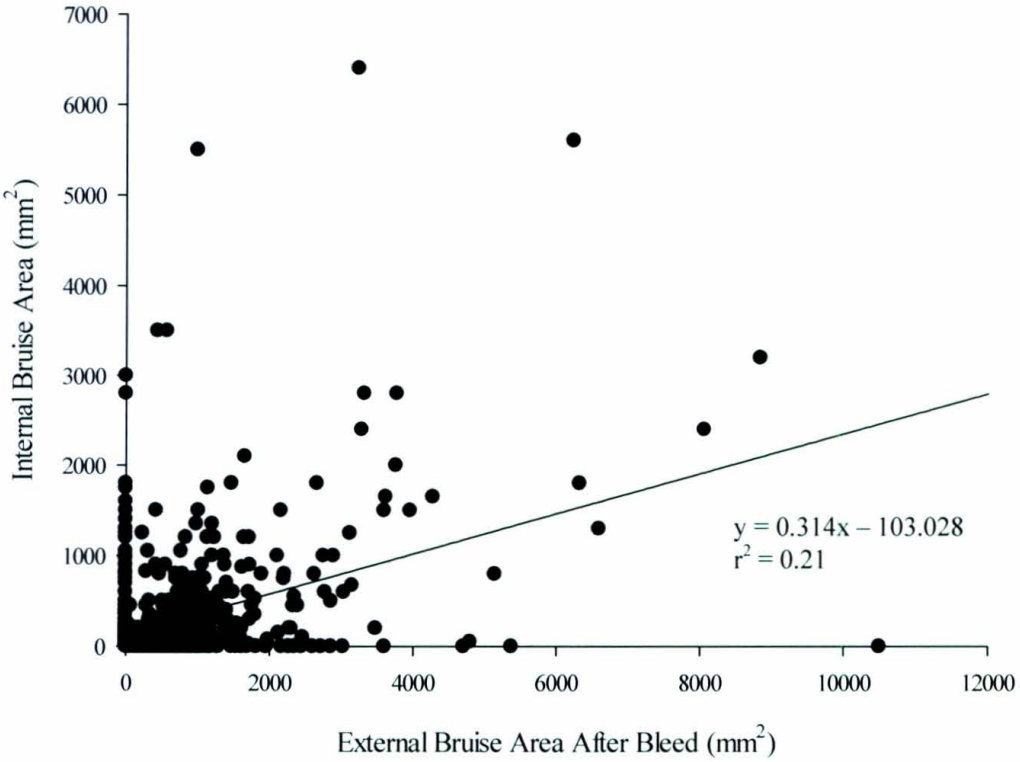


Figure 3.6 Internal area of a single bruise (mm^2) plotted by the external area of that same bruise after bleed (mm^2). Internal Bruise Area = 0.314*Bruise Area After Bleed + 103.028, $r^2 = 0.21$.

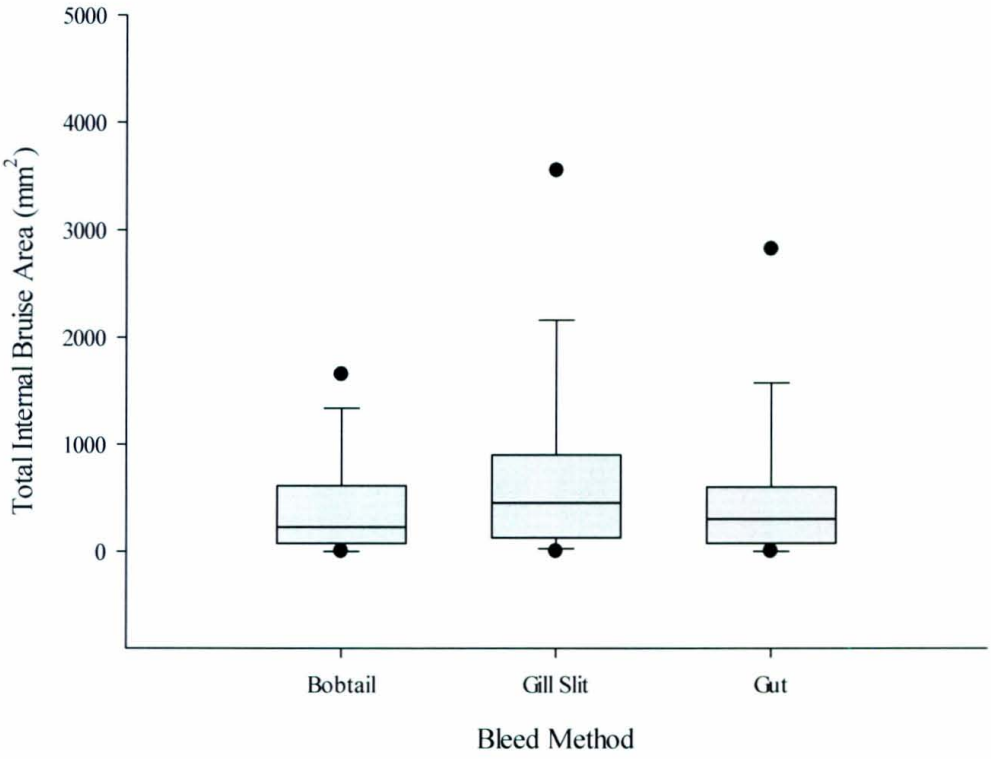


Figure 3.7 Mean internal bruise area (mm^2) for fish in the bobtail, gill slit and gut treatment groups.

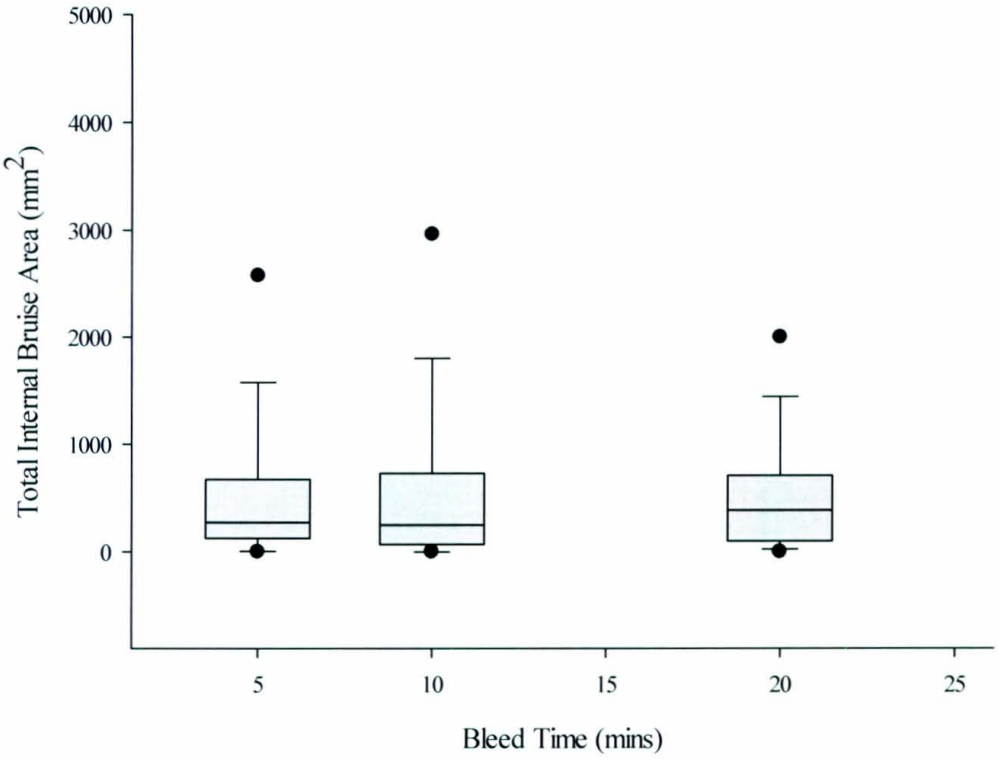


Figure 3.8 Mean internal bruise area (mm^2) for fish in the 5, 10 and 20 minute bleed time treatment groups.

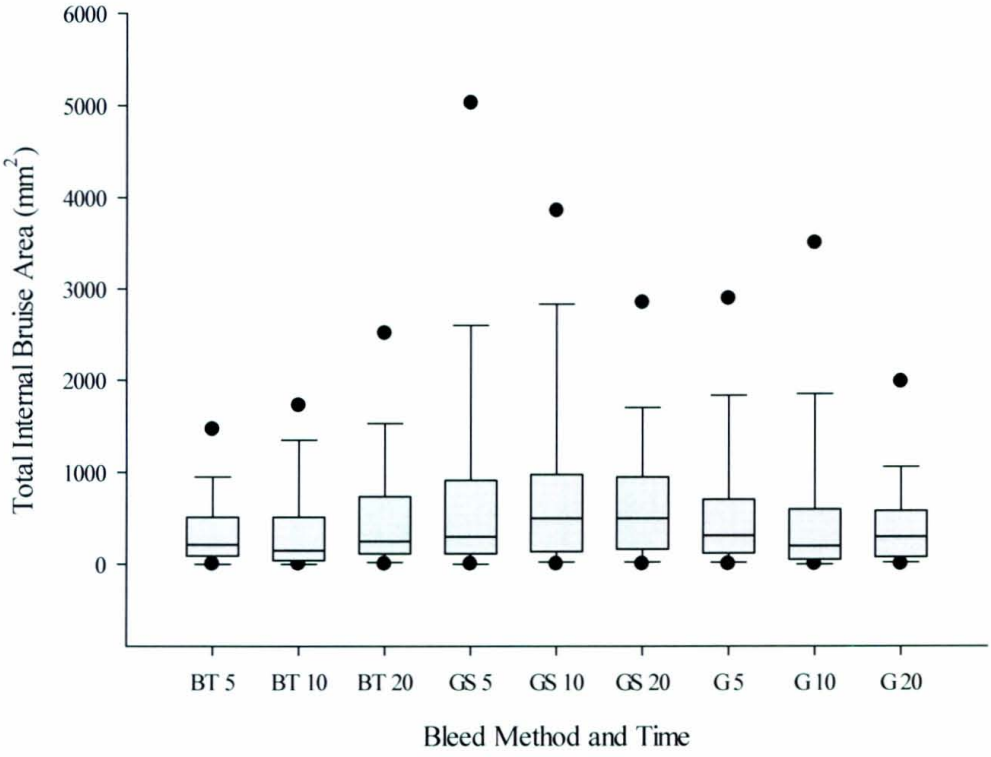


Figure 3.9 Mean internal bruise (mm^2) area of fish in each bleed time and bleed method combination treatment. BT = Bobtail, GS = Gill slit, G = Gut.

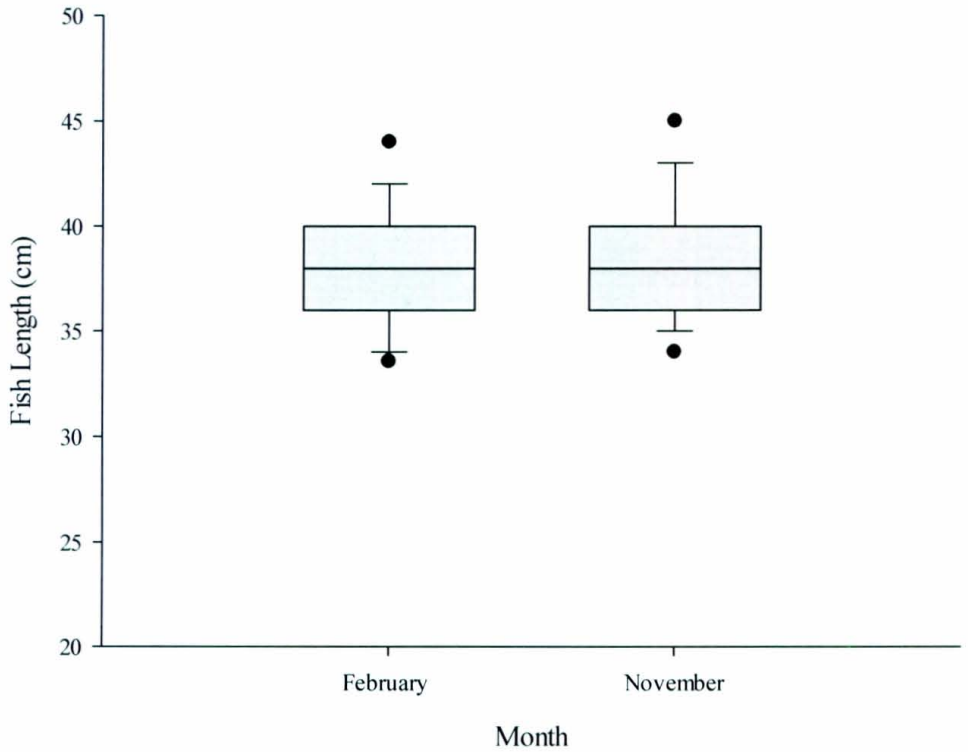


Figure 3.10 Mean fish length (cm) observed in February and November.

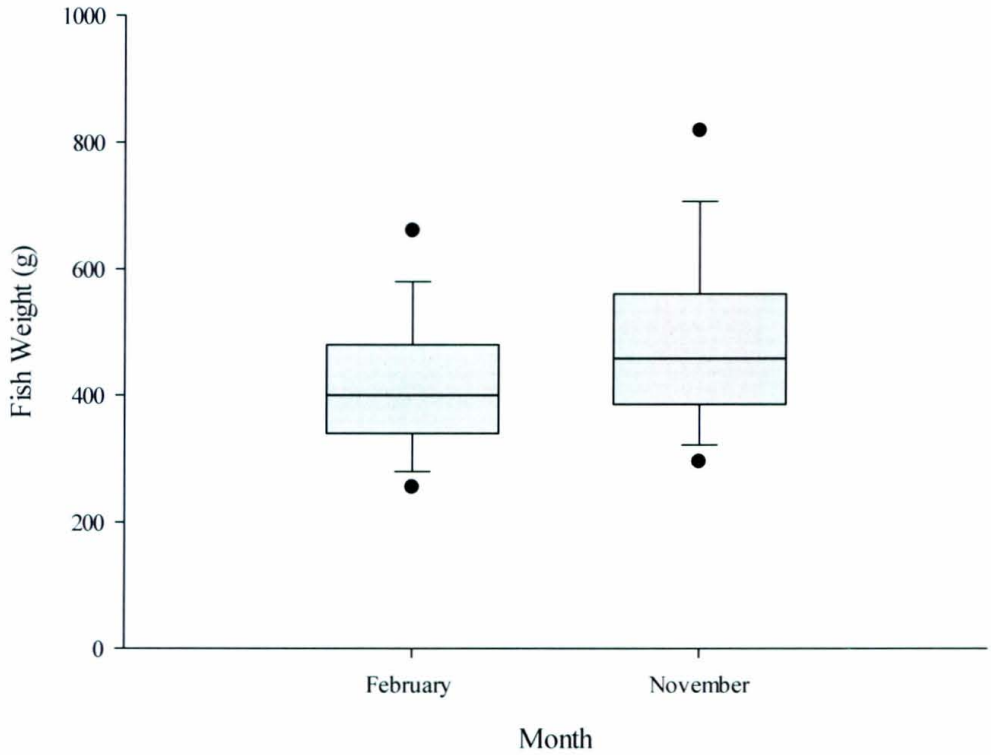


Figure 3.11 Mean fish weights (g) observed in February and November.

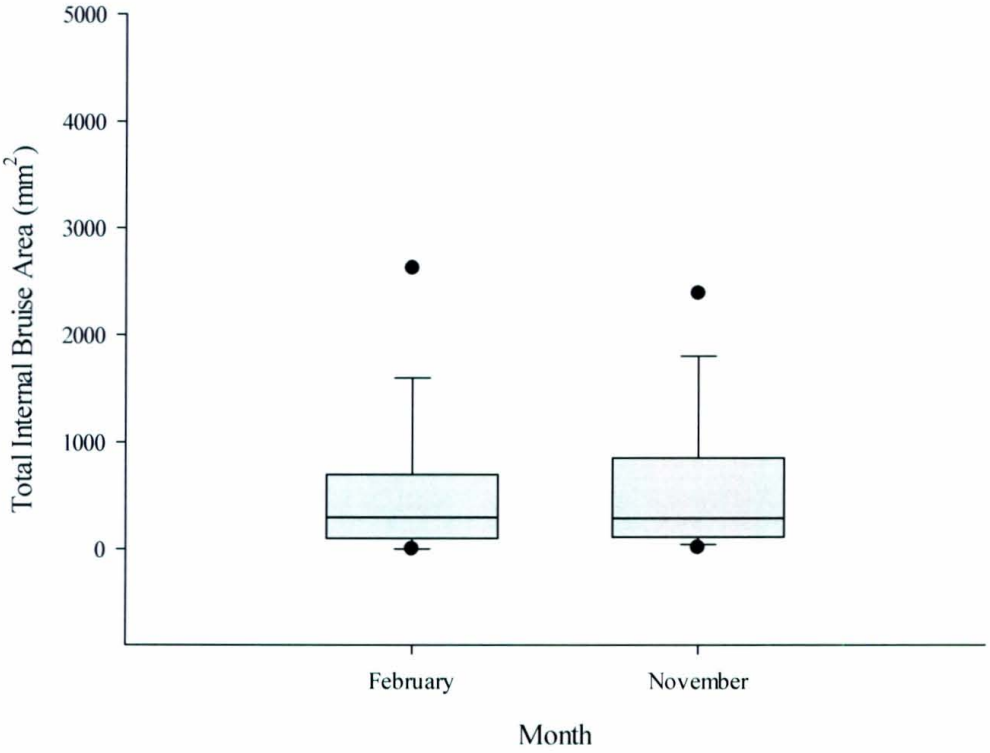


Figure 3.12 Mean fish internal bruise area (mm^2) observed in February and November.

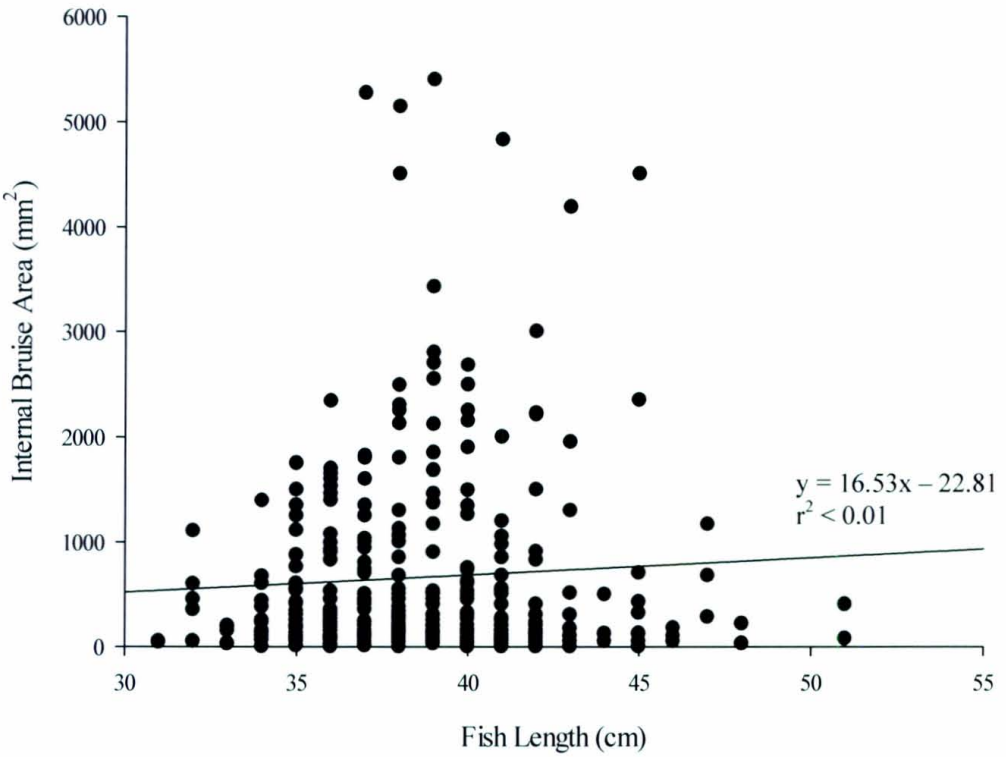


Figure 3.13 Total internal bruise area (mm^2), calculated as the sum of the areas of all the bruises measured on a given fish, plotted by fish length (cm). Internal Bruise Area = 16.53*Fish Length + 22.81. $r^2 < 0.01$.

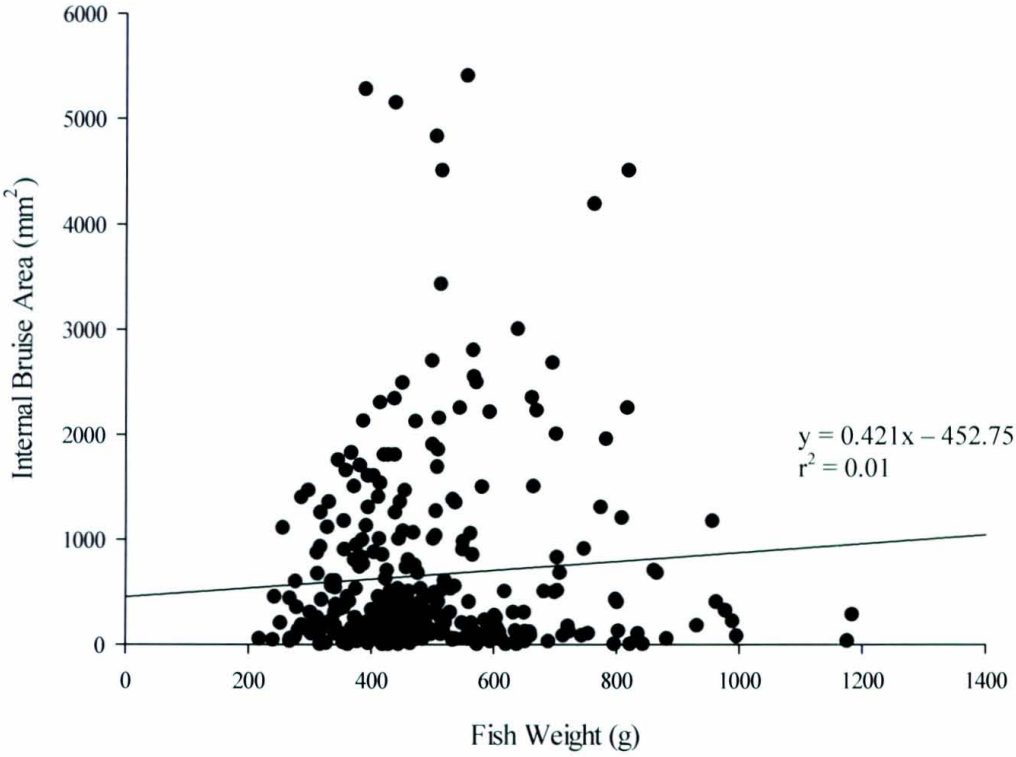


Figure 3.14 Total internal bruise area (mm^2), calculated as the sum of the areas of all the bruises measured on a given fish, plotted by fish weight (g). Internal Bruise Area = 0.421*Fish Weight + 452.75. $r^2 = 0.01$.

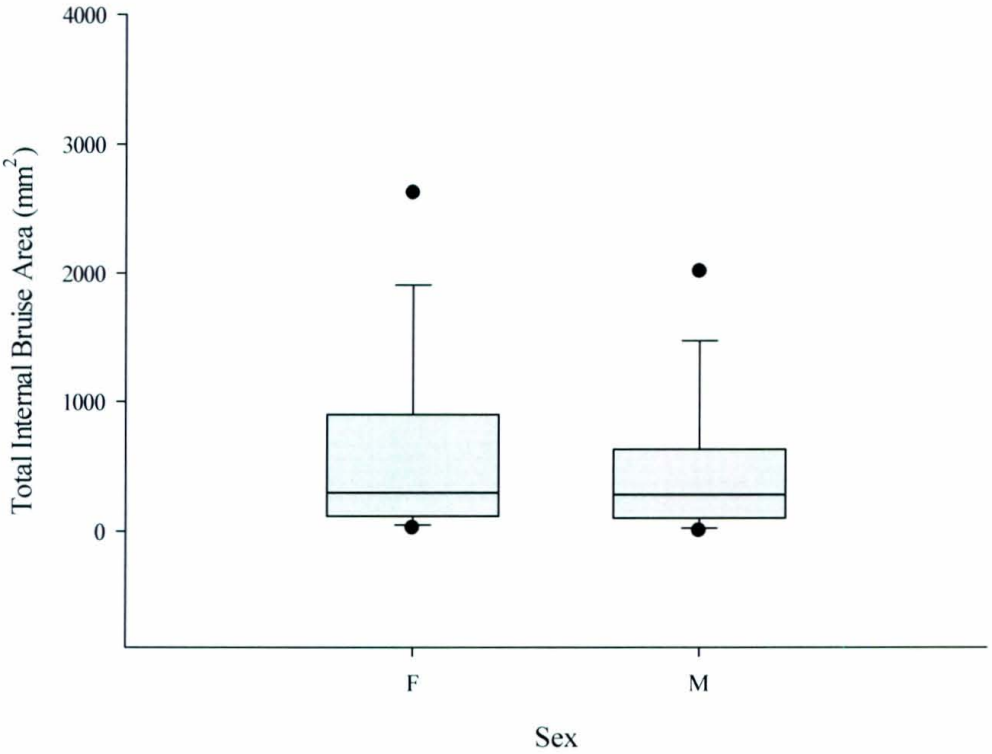


Figure 3.15 Mean internal bruise area (mm^2), calculated as the mean of the areas of all the bruises measured on a given fish, plotted by sex.

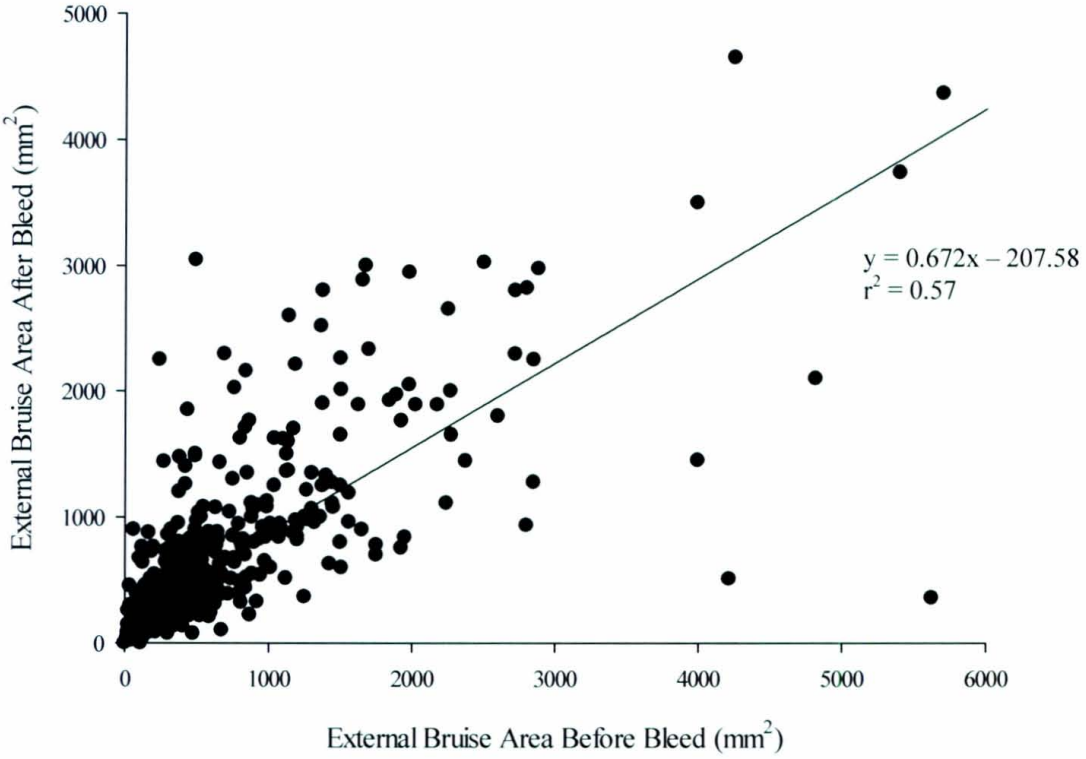


Figure 3.16 External area of a single bruise after bleed plotted by the external area of that same bruise before bleed (mm^2). External Bruise Area After Bleed = 0.672*External Bruise Area Before Bleed + 207.58, $r^2 = 0.57$.

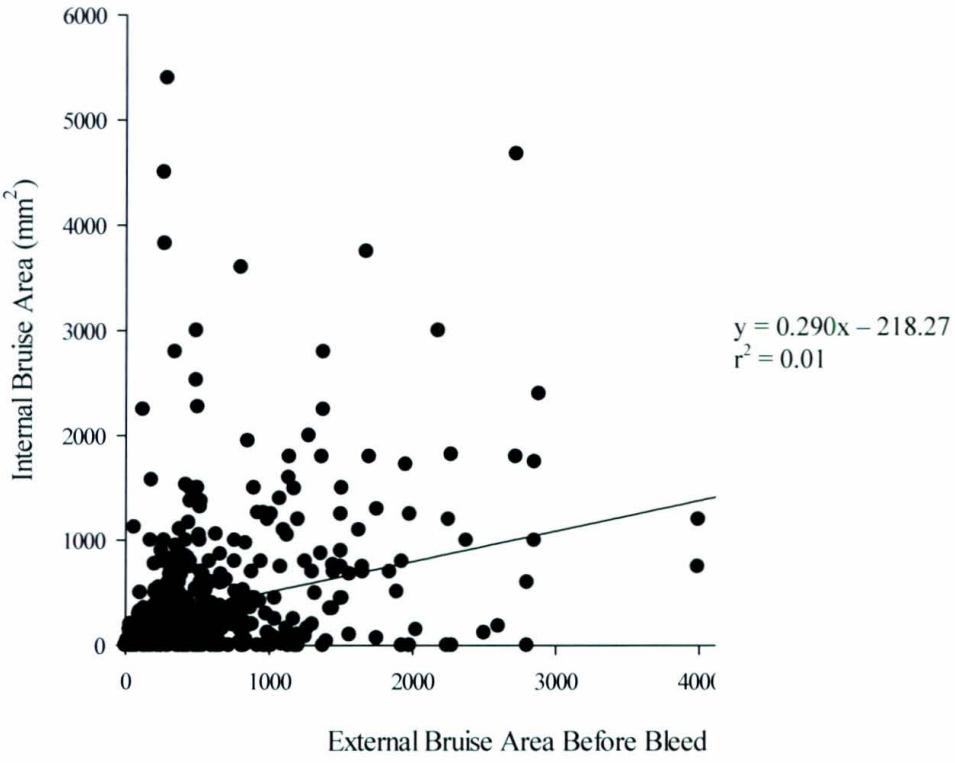


Figure 3.17 Internal area of a single bruise (mm^2) plotted by the external area of that same bruise before bleed (mm^2). Internal Bruise Area = 0.290*External Bruise Area Before Bleed + 218. 27. $r^2 = 0.11$.

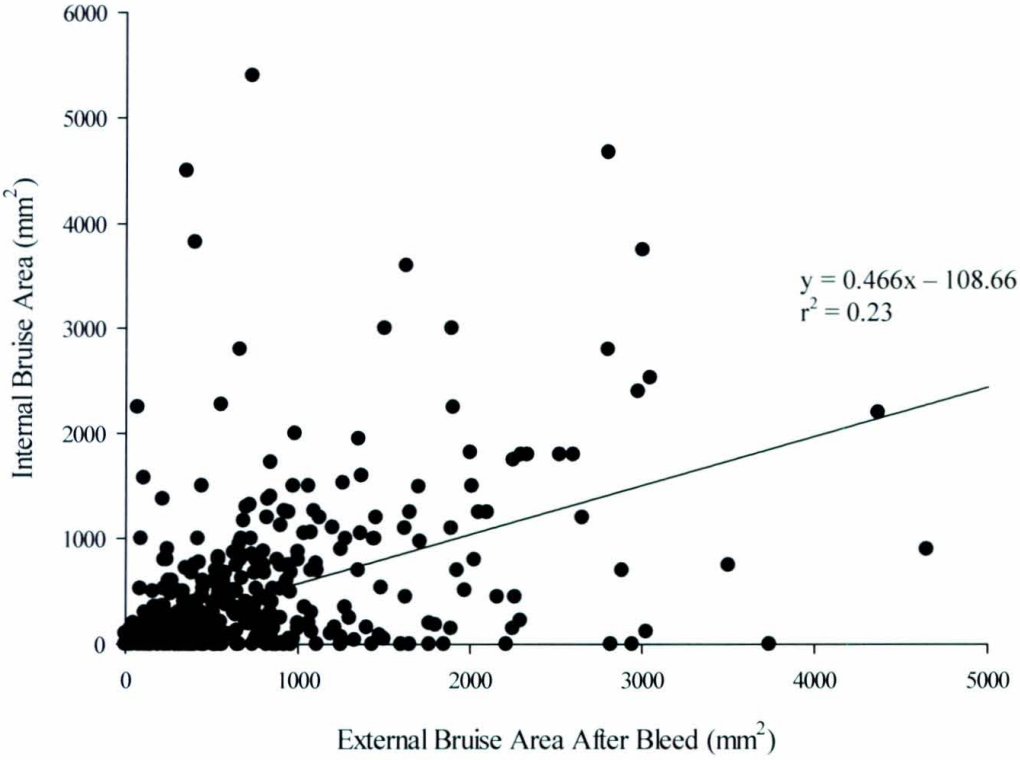


Figure 3.18 Internal area of a single bruise (mm^2) plotted by the external area of that same bruise after bleed (mm^2). Internal Bruise Area = 0.466*External Bruise Area After Bleed + 108.66, $r^2 = 0.23$.

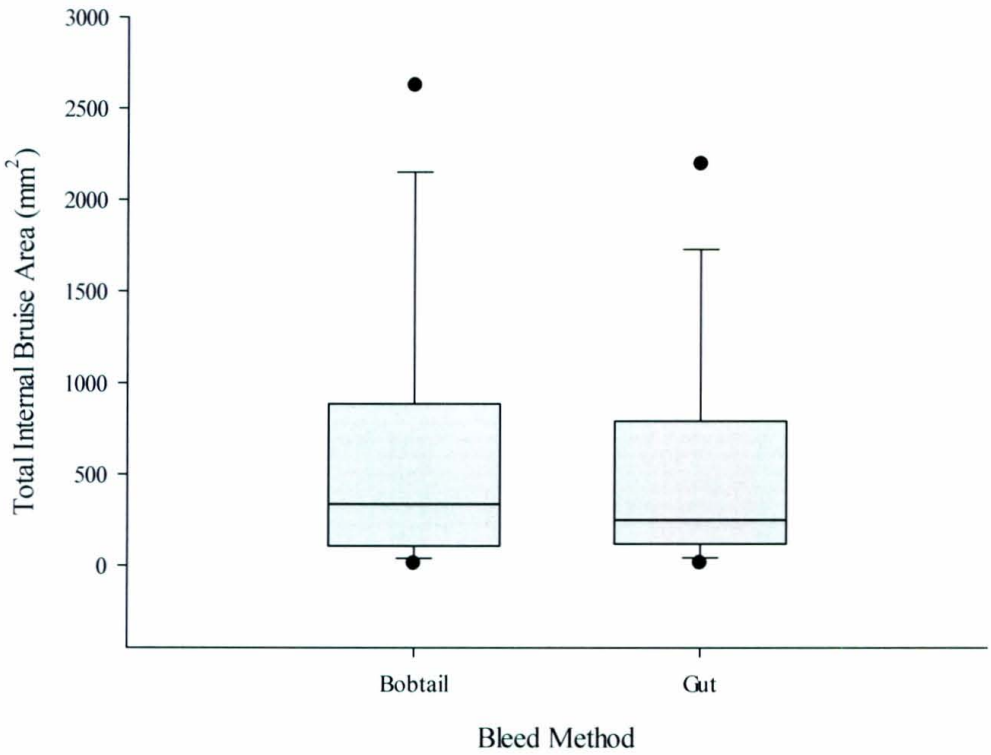


Figure 3.19 Mean total internal bruise area (mm^2) for fish bleed by bobtailing and fish bled by gutting.

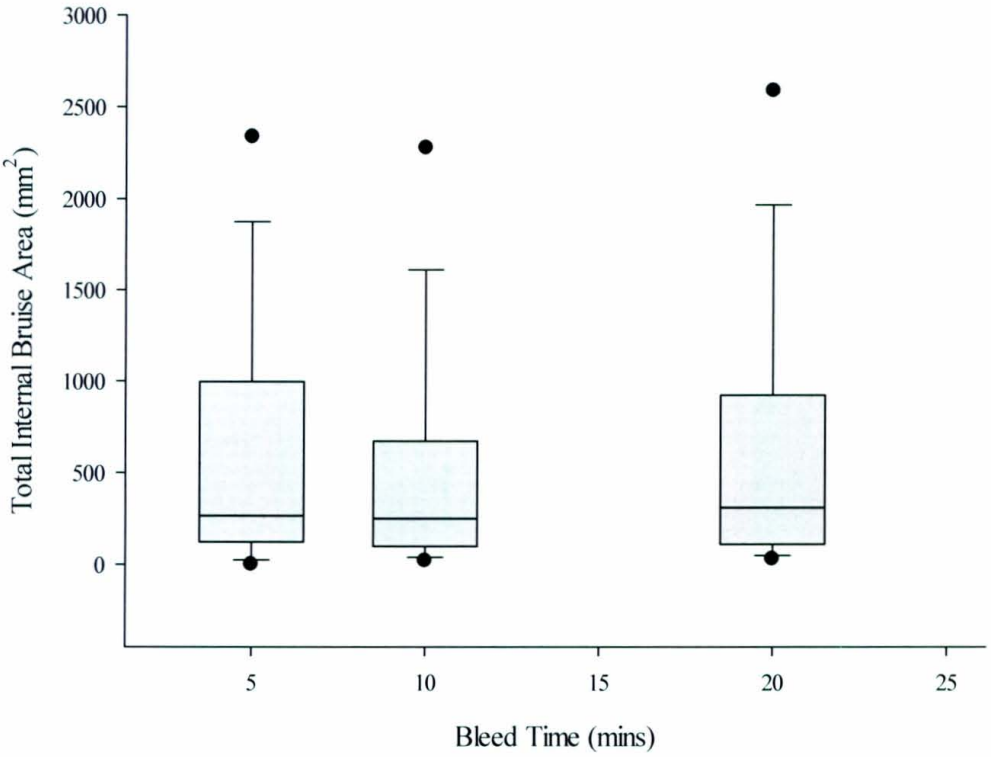


Figure 3.20 Mean internal bruise area (mm^2) for fish bled for 5, 10 and 20 minutes.

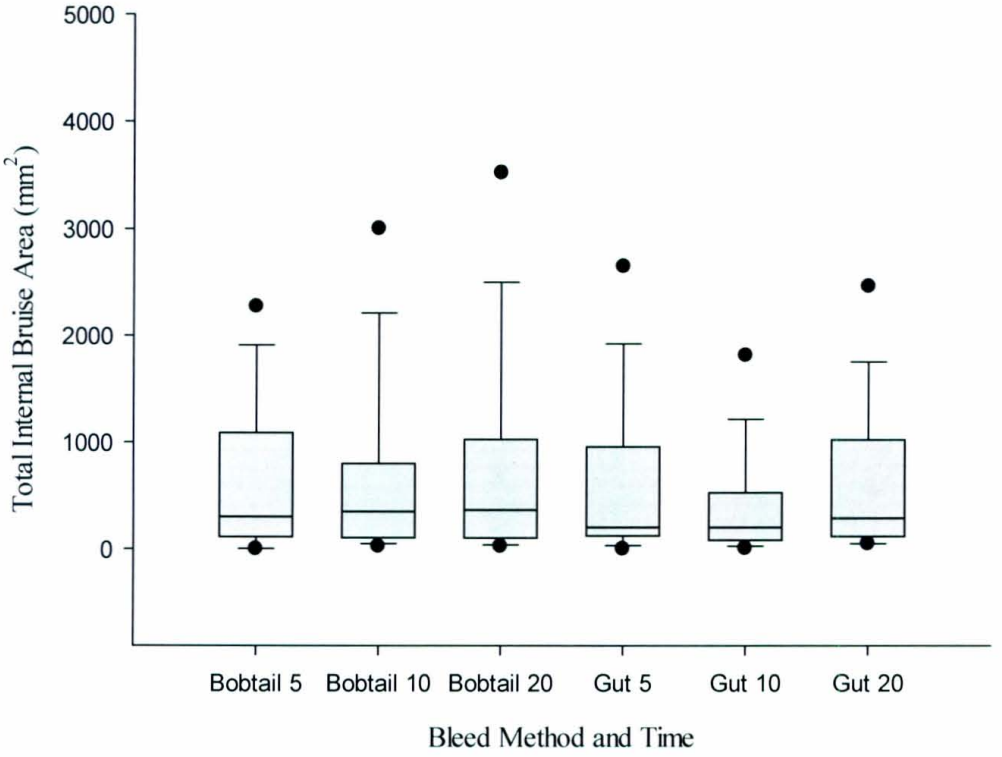


Figure 3.21 Mean internal bruise area (mm^2) of fish in each bleed time and bleed method combination treatment.

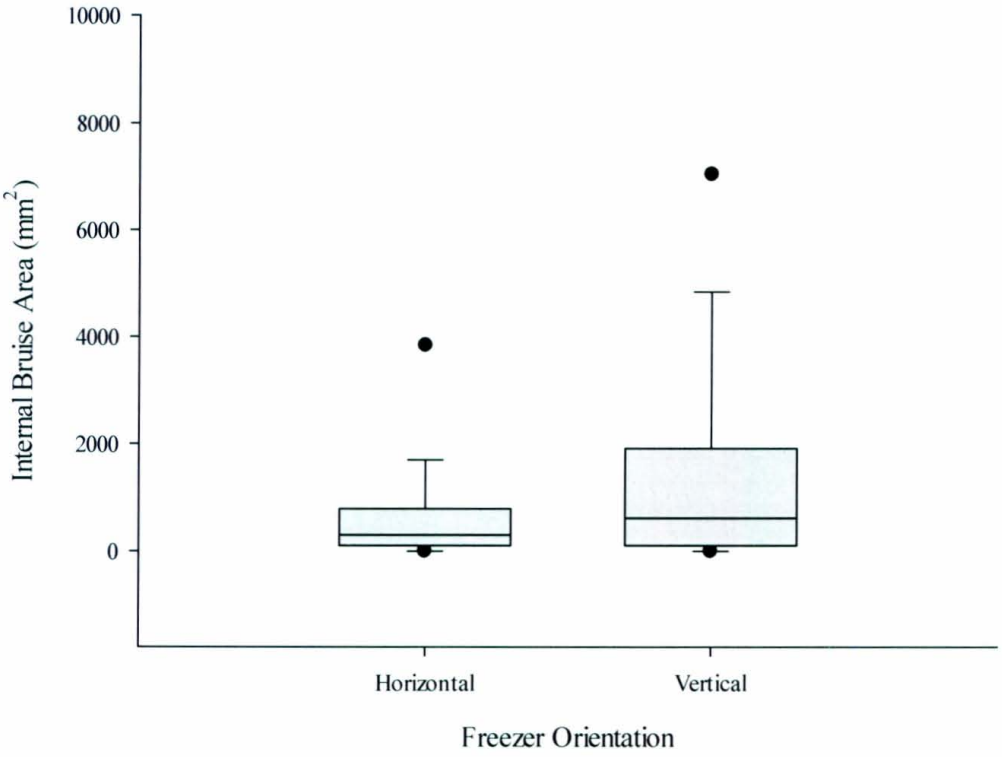


Figure 3.22 Mean internal bruise area (mm^2) of fish frozen in the horizontal plate freezer and fish frozen in the vertical plate freezer.

Chapter 4. Summary

4.1 Summary Remarks

The objective of this thesis was to characterize the discolouration patterns that are currently experienced in commercially harvested yellowtail flounder and to identify optimal processing techniques to best minimize the discolouration. Chapter 2 developed and tested innovative computer image analysis software designed to assess fillet discolouration. Results showed that bruise weight measured manually by a person was well correlated with bruise area, measured by the program. It was found that bruise area on the right fillet was not significantly different from bruise area on the left fillet. Rather interestingly however, it was found that bruises were not randomly located on the fillet, but were located predominantly at the anterior region of the fillet, and occurred in greater numbers with increasing distance from the fillet centroid. This bruise assessment program proved extremely useful both for locating bruises and obtaining their geometric and spectral properties. While this is not the first time computer image analysis software has been used to evaluate bruises and defects in fillets (e.g., Hamre *et al.* 2003; Heia *et al.* 2007; Roth *et al.* 2007; Zydlewski *et al.*; 2008, Erikson *et al.*, 2010 Rotabakk *et al.* 2011), we believe our approach was particularly novel in that it integrated sophisticated contour tracking algorithms that automatically identified, localized and measured bruises. Future use of this technology could be applied to line-scale operations at a processing plant to select and grade fillets.

Two experiments were conducted to evaluate the effects of bleed method, bleed time, and freezer orientation on the internal bruise area of yellowtail fillets (Chapter 3). Both experiments found no evidence that bruise area was affected by changes in bleed time (i.e., duration of time spent in the bleed tank). In Experiment 1, there was a difference in bruise area across bleed methods, the gill slit treatment fish having significantly more bruising than the bobtail treatment fish. For this reason, gill slitting was ruled out as the most effective bleed method and was not included in the second experiment. Both experiments showed no significant difference in bruise area between gut and bobtail treatments. Fish frozen in vertical plate freezers had significantly more bruising than those frozen in horizontal plate freezers.

The results from the study led to the recommendation that the industry continue to use the traditional processing techniques as these experiments showed no evidence to suggest any change would improve fillet quality. It was also recommended that fish should be frozen in a horizontal plate freezer, or more care should be used when loading the vertical plate freezer so as not to cause unnecessary fillet damage. External bruise area, both before and after bleeding, was also measured and compared to internal bruise area in Chapter 2. As predicted, it was found that the two measurements are correlated, but not so strongly that one should consider external bruise area reliable as a sole indicator of internal condition.

4.2 Limitations of Approach

There were a number of limitations to the approaches utilized in this thesis. In Chapter 2, the first challenge that arose was maintaining as objective an approach as possible while

manually assessing and removing bruises. To ensure as much consistency as possible throughout the data collection, one technician was responsible for all assessment and bruise removal. This minimized inter-observer discrepancy.

Another limitation to the methods used was the fact that bruise assessment and photography set up was located in the processing plant at the Marine Institute, and so had to be cleaned up entirely at the end of every day and re-set up every morning. This creates the potential for slight differences in the lighting and camera set up. Along with this, a high definition camera would have been beneficial for greater photo resolution. One other limitation to the methods in Chapter 2 is that photographs provide information about bruise area, but not volume.

In Chapter 3, the majority of the data were collected at sea, in an industry setting. This placed unique limitations on how much control we had over the research environment given that the vessel was fishing its commercial quota at the same time the research was being conducted. This meant that the experimental treatments could not unduly impede the fishing or factory activities of the vessel. While it is important to mention that the captain and crew were exceedingly willing to assist, future research initiatives might prove more effective if the vessel were chartered outside its normal fishing operations. This would mitigate competing demands, especially if difficult variables such as tow duration or haul back procedures are manipulated.

An obvious limitation of the experimental design worth noting is the fact that the "true" internal baseline condition of each individual fish fillet was not known before subjecting the live individuals to the different slaughter treatments. In my analysis, it was necessary to assume that the fish in the different treatment groups shared a similar internal condition. However, it is conceivable that individuals that seemingly looked the same during external assessment may have had very different internal conditions. This bias may have masked my ability to detect statistical differences between the treatment groups. However, in defense of the approach, it is important to note that no adequate technique currently exists and that similar experiments (e.g. Olsen *et al.*, 2006) must make similar assumptions. Future research could focus additional resources on developing a technique that definitively predicts internal fillet condition from outward live appearance, thereby allowing the researcher to be fair in his/her assignment of fish to different treatments (i.e., establishing a baseline and removing potential bias). Alternatively, simulated slaughter techniques could also be conducted under controlled laboratory conditions. The advantage of this approach is that laboratory-raised individuals would share a common history of husbandry/care and would theoretically share a common internal fillet condition.

Finally, were this experiment to be repeated, another control group could be added to include fish that were not slaughtered or bled, but just harvested and immediately flash frozen. The gutting treatment was used at the control in the experiment due to the fact that it is the industry norm, and we were interested in detecting differences between the norm and alternative methods. The inclusion of an unprocessed control group would have

established what amount of internal bruising is acquired during harvesting, and how much is reduced as a result of onboard processing. Another interesting, and perhaps more informative approach would have been to select only the most severe bruises for analyses, or to focus on bruising in the nape, as they account for the majority of the yield lost (shown in Chapter 2).

4.3 Research Initiated But Not Completed

Initial plans for this thesis included an experiment to test the effects of barotrauma on internal bruising. The experiment entailed the use of a decompression chamber to rapidly decompress yellowtail flounder in order to simulate a commercial trawling event under controlled laboratory conditions. Treatment groups were set up to test three different acclimation pressures to simulate capture depths, and three different rates of pressure removal to simulate rates of trawl ascent. If the results of this research had turned out as hypothesized, total internal bruise area would increase with increasing acclimation pressure and rate of decompression (i.e. assent). If this were the case, depending on the magnitude of the difference seen, the results of this research could have important implications for trawl retrieval protocol.

Unfortunately, a number of complications made completing the aforementioned experiment impossible. Firstly, live yellowtail flounder are a relatively difficult species to obtain. Commercially harvested (and trawled) yellowtail were basically the only option available, which meant that the condition of the fish was generally poor to begin with. Harvested fish had to be kept alive in a flow through tank onboard the vessel until return

to shore, then transported from Marystown to St. John's in an oxygenated tank, and transferred to prepared tanks in the Centre for Aquaculture and Seafood Development (CASD) at the Fisheries and Marine Institute. A number of causalities arose as a result of trawl-related injuries, but the remaining fish ended up having to be humanely euthanized due to a viral infection, similar to fin rot, that infested the tank. Secondly, numerous problems with the decompression chamber made it impossible to reach the desired pressures, and so needed to be reconstructed prior to use. In the end, complications with both the live specimens and the decompression chamber proved to be too time-consuming for the scope of this thesis and the initiative had to be abandoned until a future date.

Further research into the effects of barotrauma on internal bruising in commercially harvested fish is strongly recommended. More research on the effects of bleed method and bleed time, as well as bleed tank temperature, on internal bruise area are recommended.

4.4 Conclusion

Fillet discolouration (or bruising) is a common challenge in marine capture fisheries. This thesis aimed to develop an objective assessment technique for measuring bruises, as well as characterize the bruising patterns that are currently seen in commercially harvested yellowtail flounder (*Limanda ferruginea*). It also aimed to assess the effectiveness of bleed method, bleed time and freezer orientation to give recommendations on optimal processing techniques for minimal bruising. Major findings include that bruising occurs equally frequently on both fillets, and on both the inside and outside of the fillets. Bruises

were located non-randomly, occurring predominantly at the anterior region of the fillets. Two experiments conducted in February and November 2010 showed no evidence that fillet quality (internal bruise area) was affected by different bleeding methods or bleed times. Fish frozen in a horizontal plate freezer had less bruising than those frozen in a vertical plate freezer. These results are part of a large 3-year strategic collaborative initiative undertaken between Memorial University and Ocean Choice International.

References

Akse, L., and Joensen, S., 2004. Fangstskader på ferskt råstoff (torsk) levert fra kystflåten. Fangstskadeindeks til bruk i mottakskontroll og kvalitetssortering. *Norwegian Institute of Fisheries and Aquaculture Research*, **10** (in Norweigian).

Ando, M., Nishiyabu, A., and Makinodan, Y. 1999. Post mortem softening of fish muscle during chilled storage as affected by bleeding. *Journal of Food Science* **64**: 423-428

Barnett, C. H. 1951. The structure and function of the choroidal gland of teleostean fish. *Journal of Anatomy* ***85****:* 113-119.

Bayhan, B., Sever, T.M. and Taskavak, E. 2008. Length-weight Relationships of Seven Flatfishes (Pisces: Pleuronectiformes) from Aegean Sea. *Turkish Journal of Fisheries and Aquatic Sciences* **8**: 377-379.

Bigelow, H.W., and Schroeder, W.C. 1953. Fishes of the Gulf of Maine. US Fish Wildlife Serv. Fish. Bull. No. 74, 577p.

Botta, J.R., Squires, B.E. and Johnson, J.1986. Effect of bleeding/gutting procedures on the sensory quality of fresh raw Atlantic cod (*Gadus morhua*). *Canadian Institute of Food Science and Technology Journal* **19**:186-190.

Brodie, W.B., Walsh, S.J., and Atkinson, D.B. 1998. The effect of stock abundance on range contraction of yellowtail flounder (*Pleuronectes ferruginea*) on the Grand Bank of Newfoundland in the Northwest Atlantic from 1975 to 1995. *Journal of Sea Research* **39**:139-152.

Bruno, I., Costas, G., Gonzalez, C., and Paz, X. 2000. Feeding chronology of yellowtail

flounder (*Limanda ferruginea*) and American plaice (*Hippoglossoides platessoides*) on the Grand Bank (NAFO Division 3N). NAFO Scientific Council Studies **33**: 103-116.

Buddenbrock, W. von Henschel, J. and Friedrich, H. 1934.Untersuchung fiber das Blur und die Atmung dereinheimisehen Plattfische. I. Die morphologische Beschaffenheit des Blutes der Nordsee-, Beltsee- und Ostseescholle. Wiss. Meeresunters. Abt. Kiel **22**: 1-20.

Casillas, E. and Smith, L.S. 1977. Effect of stress on blood coagulation and hematology in Rainbow trout (Salmo gairdneri). *Journal of Fisheries Biology* **10**: 481–491.

Collette, B.B. and Klein-MacPhee G. 2002. Bigelow and Schroeder's Fishes of the Gulf of Maine (Smithsonian Press, Washington, D.C.).

Connell, J.J. 1995. *Control of Fish Quality,* 4th ed, pp. 55-61. Fishing News Books, Blackwell Science, Oxford, UK

Department of Finance of Newfoundland. 2010. The Economy 2010. http://www.economics.gov.nl.ca/E2010/Fishery.pdf. Accessed August 15th 2010.

Department of Fisheries and Aquaculture. 2011. *Seafood Industry Year in Review 2010.* http://www.fishaq.gov.nl.ca/publications/yir_2010.pdf. Accessed February 1st 2012.

Department of Fisheries and Oceans Canada. 2008. Value of Atlantic Coast Commercial Landings, By Region. http://www.dfo-mpo.gc.ca/stats/commercial/land-debarq/sea-maritimes/s2008av-eng.htm. Accessed August 15th 2010.

Department of Fisheries and Oceans Canada. 2011. Commercial Fisheries.

http://www.dfo-mpo.gc.ca/stats/commercial/sea-maritimes-eng.htm. Accessed February 2nd 2012.

Digre, H., Hansen, U.J. and Erikson, U. 2010. Effect of trawling with traditional and 'T90' trawl codends on fish size and on different quality parameters of cod *Gadus morhua* and haddock *Melanogrammus aeglefinus*. *Fish Science* **76**: 549-559.

Erikson, U., Misimi, E. and Fismen, B. 2010. Bleeding of anaesthetized and exhausted Atlantic salmon: body cavity inspection and residual blood in pre-rigor and smoked fillets as determined by various analytical methods. *Aquaculture Research* **41**: 496-510.

Food and Agriculture Organization. 1970. *Freezing at sea: advice to the crew*. Ministry of Agriculture, Fisheries and Food, Torry Research Station, Torry Advisory Note, No. 34, pp. 14.

Food and Agriculture Organization. 2010. *The State of the World Fisheries and Aquaculture 2010*. (Fisheries and Aquaculture Department, Rome).

Francis, F.J., 1995. Quality as influenced by color. *Food Quality and Prefererences* **6**: 149–155.

Fujikata A. and Y. Ikeda. 1985. Effect of handling on blood coagulation in carp. *Bulletin of the Japanese Society of Scientific Fisheries* **51**: 1093-1096

Gallagher, P., Thorarensen, H. and Farrell, A.P. 1995. Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respiratory Physiology*, **102**: 279–292

Grunwald, E.W. and Richards, M.P. 2006. Mechanisms of heme protein-mediated lipid

oxidation using hemoglobin and myoglobin variants in raw and heated washed muscle. *Journal of Agricultural Food Chemistry* **54**: 8271-8280.

Hamre, K., Lie, O. and Sadnes, K. 2003. Development of lipid oxidation and flesh colour in frozen stored fillets of Norwegian spring-spawning herring (*Clupea harengus*). Effects of treatment with ascorbic acid. *Food Chemistry* **82**: 447-453.

Hart, J. L. 1973. *Pacific fishes of Canada*. Fisheries Research Board of Canada, Ottawa.

Heia, K., Sivertsen, A.H., Stormo, S.K., Elvevoll, E., Wold, J.P. and Nilsen, H. 2007. Detection of nematodes in Cod (*Gadus morhua*) fillets by imaging spectroscopy. *Journal of Food Science* **72**(1): 11-15.

Hoar, W.S. and Randall, D.J. 1970. *Fish Physiology*, vol. 4. Academic Press, New York, USA, pp. 133–168.

Huss H.H. and Asenjo, I. 1976. Some factors inluencing the appearance of fillets from white fish.*Technological Laboratory - a report*. Ministry of Fisheries, Lyngby, Denmark, pp. 8.

Huss, H.H. 1995. Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper No. 348. Rome FAO.

Kelly, T.R. and Little, W.T. 1966. Brown discoloration in pre-rigor cut fish fillets. *Journal of Food Technology* **1**: 121-129.

Kelly, T.R. 1969. Discolouration in sea-frozen fish fillets. In: *Freezing and Irradiation of Fish* (ed. by R. Kreuzer), pp.64-67. Fishing News Books, London, UK.

Kenney, J., Rahman, W.T, Winger, W.T and Manuel, H. 2011. Quantification of bruising patterns in commercially harvested yellowtail flounder (*Limanda ferruginea),*

unpublished.

Kieley, P. "OCI restructures its operation to remain competitive in a global marketplace." Ocean Choice International, December 2nd 2011. Web January 31st 2012. http://www.oceanchoice.com/system/resources/BAhbBlsHOgZmIigyMDExLzEyLzAyLzEwXzUyXzE0XzU3M19vY2kxMjAyLnBkZg/10_52_14_573_oci1202.pdf

Maddock Parsons, D., Morgan, J., Brodie, B., and Power, D. 2011. Assessment of the NAFO 3NLO Yellowtail Flounder. NAFO SCR Doc., 11/033

Margeirsson, S., Nielsen, A.A., Jonsson, G.R. and Arason, S. 2006. Effect of catch location, season and quality defects on value of Icelandic cod (*Gadus morhua*) products. In *Seafood research from fish to dish*, ed. by J.B. Luten, C. Jacobsen, K. Bekaert, A. Saebo and J. Oehlenschlager. Wageningen Academic Publishers.

Michie, I. 2001. Causes of downgrading in the salmon farming industry. In: *Farmed Fish Quality* (ed. by S.C. Kestin & P.D. Wariss), pp. 129-136. Blackwell Science, Oxford, UK.

Misra, H.P. 1972. Generation of superoxide radical during autoxidation of hemoglobin. *Journal of Biological Chemistry* **247**: 6960–6962.

Morrissey, M., Suski, C.D., Esseltine, K.R. and Tufts, B.L. 2005. Incidence and Physiological Consequences of Decompression in Smallmouth Bass and Live-Release Angling Tournaments. *Transactions of the American Fisheries Society* **134**: 1038-1047.

Murawski, S.A., Maguire, J-J., Mayo, R.K., Serchuk, F.M. 1997. Groundfish stocks and

the fishing industry. In Boreman J., Nakashima B.S., Wilson J.A., Kendall R.L. (Eds.). Northwest Atlantic Groundfish: Perspectives on a Fishery Collapse(American Fishery Society, Bethesda, MD) pp. 27–70.

Office of the Auditor General. 2011. "Chapter 4: A study of managing fisheries for sustainability" in *Report of the commissioner of the environment and sustainable development* (Office of the Auditor General of Canada, Ottawa).

Olsen, S.H., Sorensen, N.K., Stonno, S.K. and Elvevoll, E.O. 2006. Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic Salmon (*Salmo salar*). *Aquaculture* **258**: 462-469.

Otsu, N. 1979. A threshold selection method from gray-level histograms. 1979. IEEE Transactions on Systems, Man and Cybernetics, vol. 9, no. 1, pp. 62-66.

Pelster, B. and Weber, R. E. 1991. The physiology of the Root effect. In *Advances in Comparative and Environmental Physiology*, vol. 8 (ed. R.Gilles), pp. 51-77. Berlin: Springer.

Pelster, B. and Randall, D. J. 1998. The physiology of the Root effect. In *Fish Respiration* (ed. S. F. Perry and B. L. Tufts), pp. 113-139. San Diego: Academic Press.

Pitt, T.K. 1970. Distribution, abundance, and spawning of yellowtail flounder, *Limanda* ferruginea, in the Newfoundland area of the Northwest Atlantic. *Journal of Fisheries Research Board Canada* **27**: 2261-2271.

Puppo, A. and B. Halliwell. 1988a. Formation of hydroxyl radicals from hydrogen-peroxide in the presence of iron — is hemoglobin a biological fenton reagent. *Biochemistry Journal* **249**: 185–190.

Puppo, A. and B. Halliwell. 1988b. Formation of hydroxyl radicals in biological-systems — does myoglobin stimulate hydroxyl radical formation from hydrogen-peroxide. *Free Radicals Research Communication* **4**: 415–422.

Richards, M.P. and H.O. Hultin. 2002. Contributions of blood and blood components to lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry* **50**: 555-564.

Robb, D.H.F., Phillips, A.J. and Kestin, S.C. 2003. Evaluation methods for determining the prevalence of blood spots in smoked Atlantic Salmon and the effect of exsanguinations method on prevalence of blood spots. *Aquaculture* **217**: 125-138.

Rotabakk, B.T., Skipnes, D., Akse, L. and Birkeland, S. 2011. Quality assessment of Atlantic cod (*Gadus morhua*) caught by longlining and trawling at the same time and location. *Fisheries Research* **112**: 44-51.

Roth, B., Torrissen, O.J. and Slinde, E. 2005. The effect of slaughtering procedures on blood spotting in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture* **250**: 796-803.

Roth, B., Schelvis-Smit, R., Stien, L.H., Foss, A., Nortvedt, R., and Imsland, A. 2007. Exsanguination of turbot and the effect on fillet quality measured mechanically by sensory evaluation, and with computer vision. *Journal of Food Science* **72**(9): 525-531.

Roth, B., Obach, A., Hunter, D., Nortvedt, R. and Oyarzum, F. 2009. Factors affecting residual blood and subsequent effect on bloodspotting in smoked Atlantic salmon. *Aquaculture* **297**: 163-168.

Ruff, N., Fitzgerald, R.D., Cross, T.F., Hamre, K. and Kerry, J.P. 2003. Assessment of dietary vitamins Ea nd C on market-size turbot (*Scophthalmus maximus)* fillet quality. *Aquaculture Nutrition* **9**: 91-103.

Ruis, M.A.W. and C.J. Bayne. 1997. Effects of acute stress on blood clotting and yeast killing by phagocytes of rainbow trout. *Journal of Aquatic Animal Health* **9**:190-195.

Schill, D.J. and Elle, F.S. 2000. Healing of electroshock-induced hemorrhages in hatchery Rainbow Trout. *North American Journal of Fisheries Management* **20**: 730-736.

Smit, G.L. and Schoonbee, H.J. 1988. Blood coagulation factors in the freshwater fish *Oreochromis mossambicus*. *Journal of Fish Biology* **32**: 673-677

Thorarensen H., Gallaugher, P.E., Kiessling, A.K. and Farrell, A.P. 1993 Intestinal blood flow in swimming Chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *Journal of Experimental Biology* **179**:115-129.

Tretsven, W.I. and Patten, B.G. 1981. Effect of arterial incisions on the amount of bleeding and flesh quality of rainbow trout. *Marine Fisheries Review* **43**:16-18.

Valdimarsson G., Matthiasson, A. and Stefansson, G. 1984. The effect of on board bleeding and gutting on the quality of fresh, quick frozen and salted products. In: FiftyYears of Fisheries Research in Iceland (ed. by A. Moller), pp. 61-72. Icelandic Fisheries Laboratory, Reykjavik, Iceland.

Wagenknecht, G. 2007. A contour tracing and coding algorithm for generating 2D contour codes from 3D classified objects. *Pattern Recognition* **40**(4): 1294-

1306.

Walsh, S.J. 1991. Diel variation in ability and vulnerability to avoid survey a trawl. *Journal of Applied Icthyology* **7**: 147-159.

Walsh, S.J., Veitch, M.F., Brodie, W.B., and Dwyer, K.S. 2004. Distribution and abundance of *yellowtail* flounder (*Limanda ferruginea*) on the Grand Bank, in NAFO Divisions 3LNO, from Canadian bottom trawl surveys in 1984-2003. NAFO Scientific Council of Research Doc. 04/36, 50p.

Warriss P.D. and Wilkins L.J. 1987. Exsanguination of meat animals. In: *Pre-slaughter Stunning of Food Animals* (ed. by H.E. Carter & V.R. Carter), pp. 150-158. Royal Society for Prevention of Cruelty to Animals, Horsham, Sussex, UK.

Wellman, J. 2009. The Burin Peninsula – A 2009 Snapshot. *The Navigator* **12**(6): 28-30.

Winger, P.D. 2008. Fishing smarter: improving harvesting through the study of animal behavior. *Journal of Ocean Technology* **3**(2): 15-20

Winger, P.D., Eavrs, S., and Glass, C.W. 2010. Fish behavior near bottoms trawls. *In:* Behaviour of Marine Fishes: Capture Processes and Conservation Challenges. Edited by P. He. Blackwell Publishing. pp 67-103.

Wittenberg, J. B. and Wittenberg, B.A. 1974. The choroid rete mirabile of the fish eye, I. Oxygen secretion and structure: comparison with the swim bladder rete mirabile. *Biological Bulletin* **146**:116–136.

Zydlewski, G.B., Gale, W. and Holmes, J. 2008. Use of electroshock for euthanizing and immobilizing adult spring Chinook salmon in a hatchery. *North American Journal of Aquaculture* **70**: 415-424.