

Lokaskýrsla R 03205

Skilgreining kjöreldisaðstæðna og þróun nýrra framleiðsluaðferða í sandhverfueldi

Biological optimization and development of processing methods for turbot farming

PART 1 – COMMERCIAL SCALE VALIDATION OF LONG TERM EFFECTS OF TEMPERATURE-STEP REARING ON GROWTH, FEED CONVERSION EFFICIENCY AND BLOOD PHYSIOLOGY IN TURBOT	1
1. PART 1. INTRODUCTION	1
2. PART 1. MATERIALS AND METHODS.....	2
2.1 PRE-EXPERIMENTAL PROTOCOL.....	2
2.2 EXPERIMENTAL DESIGN.....	2
2.3 GROWTH MEASUREMENTS	3
2.4 BLOOD PHYSIOLOGY.....	4
2.5. STATISTICAL METHODS.....	4
3. PART 1. RESULTS.....	4
3.1 GROWTH.....	4
3.2. FEEDING RATE AND FEED CONVERSION EFFICIENCY.....	6
3.3 BLOOD PHYSIOLOGY.....	7
4. PART 1. DISCUSSION	9
CONCLUDING REMARKS AND INCREASED VALUE OF SEA PRODUCTS	10
5. PART 1. ACKNOWLEDGEMENTS.....	10
6. PART 1. REFERENCES.....	11
PART 2 – SLAUGHTER QUALITY AND RIGOR CONTRACTION IN FARMED TURBOT: A COMPARISON BETWEEN DIFFERENT STUNNING METHODS.....	14
1. PART 2. INTRODUCTION.....	14
2. PART 2. MATERIAL AND METHODS.....	15
2.1 GENERAL.....	15
2.2 EXPERIMENTAL PROTOCOL	16
2.3 STATISTICAL ANALYSIS.....	18
3. PART 2. RESULTS.....	18
3.1 GENERAL.....	18
3.2 RIGOR MORTIS.....	19
3.3 MUSCLE pH.....	20
3.4 TEXTURE HARDNESS AND SHEAR FORCE.....	21
4. PART 2. DISCUSSION.....	23
CONCLUDING REMARKS AND INCREASED VALUE OF SEA PRODUCTS	25

5. PART 2. ACKNOWLEDGEMENTS.....	25
6. PART 2. REFERENCES.....	26
PART 3 – EXSANGUINATION OF TURBOT AND THE EFFECT ON FILLET QUALITY MEASURED MECHANICALLY, SENSORY AND WITH COMPUTER VISION.....	29
1. PART 3. INTRODUCTION.....	29
2. PART 3. MATERIALS AND METHODS.....	30
2.1 EXPERIMENT 1: SENSORY AND VISUAL APPEARANCE OF BLOOD MEASURED BY TASTE PANEL AND COMPUTER IMAGING SYSTEM (ICELAND).....	30
2.2 EXPERIMENT 2: SENSORY AND TEXTURE ANALYSIS OF BLED AND UNBLED FISH (PORTUGAL).....	31
2.3 COMPUTER IMAGING ANALYSIS.....	31
2.4 TEXTURE ANALYSIS	32
2.5 SENSORY ANALYSIS.....	32
2.6 STATISTICAL ANALYSIS.....	33
3. PART 3. RESULTS.....	33
3.1 COMPUTER IMAGING SYSTEM.....	33
3.2 TEXTURE ANALYSIS.....	34
3.3 SENSORY ANALYSIS.....	35
4. PART 3. DISCUSSION	37
CONCLUDING REMARKS AND INCREASED VALUE OF SEA PRODUCTS	39
5. PART 3. ACKNOWLEDGEMENTS.....	39
6. PART 3. REFERENCES.....	40

PART 1 - Commercial scale validation of long term effects of temperature-step rearing on growth, feed conversion efficiency and blood physiology in turbot

Summary R 032 05 – Part 1

The aim of this study was to investigate the possible benefit of “temperature-steps” rearing for juvenile turbot (initial weight 15.1 g) under realistic production conditions. One group (called 22-19-16) of juvenile turbot was reared at three different temperatures i.e. 22°C (from 15-60 g) followed by 19°C (from 60-100 g) and 16°C (> 100 g), another group (called 19-16) at two temperatures i.e. 19°C (from 15-100 g) and lowered to 16°C (> 100 g) and the third group (called 16) at one constant temperature i.e. 16°C. Growth was significantly higher in the two temperature-step groups and final mean weight was 16-18% larger in the two temperature-step groups compared to the constant temperature group. Feed conversion efficiency was highest in the 19-16 group reflecting the overall differences seen in mean weight for the three experimental groups. Only minor effects of the experimental rearing on blood physiology were found, with one notable exception of inverse relationship between plasma glucose and growth. Overall, these findings indicate that a short interval of rearing fish at high temperature during the early juvenile phase may have a long term effect on biomass increment in turbot. This is an important finding for the turbot industry.

Skýrsluágríp R 032 05 – Hluti 1

Tilgangur tilraunarinnar var að kanna hugsanlegan ávinning af því að ala sandhverfuseiði (upphafsþyngd 15.1 g) á “hitastigströppum” við raunverulegar framleiðsluaðstæður. Einn tilraunahópur (hér nefndur 22-19-16) sandhverfuseiða var alinn við þrjú mismunandi (og stíglækkandi hitastig) þ.e. fyrst við 22°C (frá 15-60 g), síðan við 19°C (frá 60-100 g) og að endingu við 16°C (> 100 g). Annar tilraunahópur (hér nefndur 19-16) var alinn við tvö hitastig þ.e. 19°C (frá 15-100 g) og 16°C (> 100 g). Þriðji hópurinn (nefndur 16) var alinn á 16°C í gegnum allt framleiðsluferlið. Þessir þrír hópar voru aldir þrjú mánuði á mismunandi hitastigum, en síðan voru þeir aldir saman í níu mánuði.

Vöxtur var marktækt hærri í báðum hitastigströppuhópunum og var lokþyngd 16-18% hærri í hitastigströppuhópunum samanborið við hópinn á stöðugu hitastigi. Fóðurnýtni var best í 19-16 hópnum og sýndi marktæka fylgni við þyngdaraukningu sama hóps. Aðeins fundust smávægileg áhrif eldishitastigs á lífeðlisfræðilegar breytur mældar í blóði. Undantekning frá þessu var marktækt samband á milli plasma glúkósa og vaxtar. Í heildina benda gögnin til þess að hægt sé að auka vöxt sandhverfu verulega með því að ala hana við stíglækkandi hitastig í stað stöðugs eldishitastigs. Þetta eru mikilvægar upplýsingar fyrir sandhverfuframleiðendur.

PART 1 – Commercial scale validation of long term effects of temperature-step rearing on growth, feed conversion efficiency and blood physiology in turbot

1. PART 1. Introduction

In juvenile turbot *Scophthalmus maximus* growth rate is significantly influenced by temperature, following a pattern typical of most fish species (cf. Imsland and Jonassen, 2002). Fish typically show a rapid increase in relative growth rate as the temperature rises, passing through a peak at optimum temperature ($T_{opt}G$) and falling rapidly at temperatures beyond $T_{opt}G$ (cf. Imsland et al., 1996, 2000, 2006). A common finding in studies examining the relationship of temperature and size on growth is that $T_{opt}G$ shifts to lower temperatures as fish increase in size. The findings of different temperature optima for different size classes together with the downward trend of the $T_{opt}G$ with size can be summarized in what we call the “stepwise-temperature-hypothesis”. Instead of using constant rearing temperatures, one utilizes specific “temperature-steps” where the fish are reared at optimum temperatures defined for each size class i.e. temperature should be lowered following changes in fish size, mimicking a mechanism suggested for wild turbot. Déniel (1990) reported that juvenile turbot stay in coastal waters, and migrate to deeper and cooler waters when reaching maturity. Other studies have confirmed that adult turbot migrate to deeper waters than do juvenile turbot (Aneer and Westin 1990; Iglesias and Rodríguez-Ojea, 1994). An ontogenetic shift in optimal temperature for growth has, thus, consequences for the natural distribution of different life stages of that species as well as for rearing under culture conditions. The $T_{opt}G$ for juvenile turbot is highly size dependent and drops rapidly in the first 6-8 months of the juvenile period. For juveniles < 50 g $T_{opt}G$ is reported to be from 20 to 22°C (Imsland et al., 2000, 2001), for 50-100 g juveniles $T_{opt}G$ is reported around 19°C (Burel et al., 1996; Imsland et al., 1996), and for juveniles > 100 g $T_{opt}G$ is reported 16-17°C (Imsland et al., 2006). In the present study we try to mimic this ontogenetic drop in $T_{opt}G$ by rearing fish at different temperature-steps and comparing growth, feed conversion efficiency and blood physiology in these groups. One group of juvenile turbot was reared at three different temperatures i.e. 22°C followed by 19°C and 16°C, another group at two temperatures i.e. 19°C and lowered to 16°C and the third group at one constant temperature i.e. 16°C. There are two new aspects in this trial compared to the published literature. Firstly, the long term follow-up of individually tagged fish and

secondly the implementation at a commercial farm level as the comparison was done under realistic production scale for a large part of a production cycle.

2. PART 1. Materials and methods

2.1 Pre-experimental protocol

Juvenile turbot of mixed parental background were used in this experiment. The eggs were spawned and hatched at Icelandic Marine Research Institute, Grindavik, Iceland, and the larvae and juveniles reared under intensive conditions. In February 2005, a batch of approximately 5200 turbot juveniles were brought to the commercial turbot farm Sæbýli, SW-Iceland and reared at 17-18°C and continuous light in one tank (bottom area 10 m²) until tagging. The fish was hand fed twice daily with formulated dry feed (Dan-Ex 1562). Pellet size 3 mm was used in at the start of the experiment, with gradual introduction of bigger pellets according to fish size and producers recommendations. Tanks were supplied with seawater (30‰) pumped from boreholes. To maintain the desired temperature a heat exchanger was used to regulate the inlet water temperature to each of the tanks. To maintain sufficient oxygen level in the tanks the inlet water into each tank was oxygenated with liquid oxygen. Oxygen saturation was higher than 80% at all times.

2.2 Experimental design

The experiment was carried out from 12 April 2005 until 11 April 2006. Initially the fish were distributed into three rectangular tanks. Two tanks had bottom area of 10 m² and one tank 14 m². Fish number in the three tanks was adjusted so initial density was similar in all tanks (1.5 kg/m²). On 5 April 118–120 fish in each tank (a total of 356 fish) were tagged intraperitoneally with Trovan tags and distributed randomly into the three tanks. One treatment group remained at 16°C (14 m² tank), one group was reared at 22°C (temperature raised in two days, 10 m² tank) and the third one was reared at 19°C (10 m² tank). On 18 June the temperature of the 22°C group was lowered to 19°C. These three experimental groups are called: 16, 19-16 and 22-19-16. Temperature for the three groups varied with (\pm S.D) 0.4, 0.5 and 1.7°C, respectively of that prescribed in the experimental period from April –July (Fig. 1). Due to mechanical failure temperature in all three groups was lowered to 11°C from 18 July to 18 August.

In late August 2005 all fish were transferred to another commercial farm (Silfurstjarnan, NE-Iceland) and reared in two commercial size tanks (bottom area 11 m²) with

identical rearing regimes. Here all groups were reared together in two tanks with identical temperature regime. Mean temperature in the rearing period at Silfurstjarnan (September 2005-April 2006) was 15.7°C (SD, 1.7°C) (Fig. 1). Flow rate into the tanks was kept at a level to sustain oxygen saturation higher than 80% in the effluent water. In February 2006 the turbot were transferred to larger tanks (16 m²).

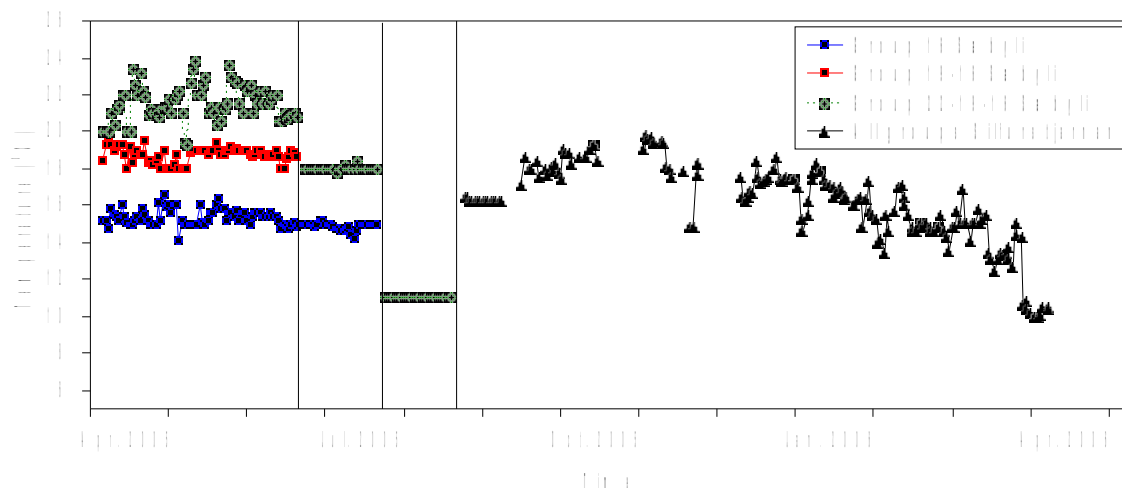


Fig. 1. Temperature during the whole experimental period for the three experimental groups at Sæbyli and at Silfurstjarnan farm (all tagged fish reared in the same tank). The first vertical dark line marks the lowering of temperature of group 22-19-16 to same level as 19-16 group, the second vertical line marks when all groups were put on same temperature regime at Sæbyli and the third vertical line marks transfer of the turbot to Silfurstjarnan farm.

2.3 Growth measurements

On the 12 April 2005 the weight of the individually tagged fish was recorded. The initial mean weight (\pm S.D) was 15.1 (\pm 4.1) g and there was not a significant (One way ANOVA, $P = 0.695$) difference in weight between temperature groups. The tagged fish were weighed individually at a monthly interval (April–July 2005) and later at monthly to bimonthly intervals (September 2005–April 2006). During each measurement the number and total weight of the untagged fish in each group was recorded so that the total biomass in each tank could be calculated. Water temperature and oxygen level (%) for each tank was measured and recorded every morning and the amount of feed fed was recorded on a daily basis. Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981):

$$\text{SGR} = (e^g - 1) 100$$

where $g = (\ln(W_2) - \ln(W_1)) (t_2 - t_1)^{-1}$ and W_2 and W_1 are weights on days t_2 and t_1 , respectively.

Feed conversion efficiency (FCE) was calculated as biomass gain per weight unit of

consumed feed:

$$FCE = (B_2 - B_1) / C$$

where, C is feed consumption (g dry matter) in the period and B_1 and B_2 are fish biomass (g wet weight) on days t_1 (start) and t_2 (final) respectively. The fish were hand fed to satiation and C calculated from amount feed given in each period. Data was not available for two periods during the trial period (e.g. July 2005 – Sept. 2005, Feb. 2006 – April 2006, see Table 1).

2.4 Blood physiology

To monitor the possible effect of different temperature regimes on blood physiology, blood samples were collected from the caudal vessels of eight individually tagged fish from each experimental group in July 2005 and April 2006 and analysed using an i-STAT Portable Clinical Analyzer. The analyzer was used in conjunction with EC8+ disposable cartridges, measuring blood sodium, potassium content, glucose, haematocrit, pH level, partial pressure of CO_2 (pCO_2), and displaying calculated values of blood bicarbonate content, total carbon dioxide concentration and haemoglobin.

2.5. Statistical methods

One-way ANOVA (Zar, 1984), where was applied to calculate the effect of different temperature regimes on mean weights, SGR and FCE. Two-way analysis of covariance (ANCOVA), where the fish weight was used as a covariate and the temperature regimes as independent variable, was applied to calculate the effect of different experimental regimes on blood physiology. In case of significant ANOVA and ANCOVA's Student-Newman-Keuls multiple comparison tests were used to locate differences among treatments (Zar, 1984). Individual growth trajectories were analysed using a growth curve analysis model (GCM, Timm, 1980). A significance level (α) of 0.05 was used if not stated otherwise.

3. PART 1. Results

3.1 Growth

From day 36 and throughout the experimental period the two temperature-step groups (i.e. 19-16 and 22-19-16) displayed overall higher mean weights compared to the 16 group (ANOVA, $P < 0.05$, Fig. 2). The two temperature-step groups maintained their weight advantage throughout the experimental period (Fig. 2). Final mean weight was 16-18% larger

in the two temperature-steps groups.

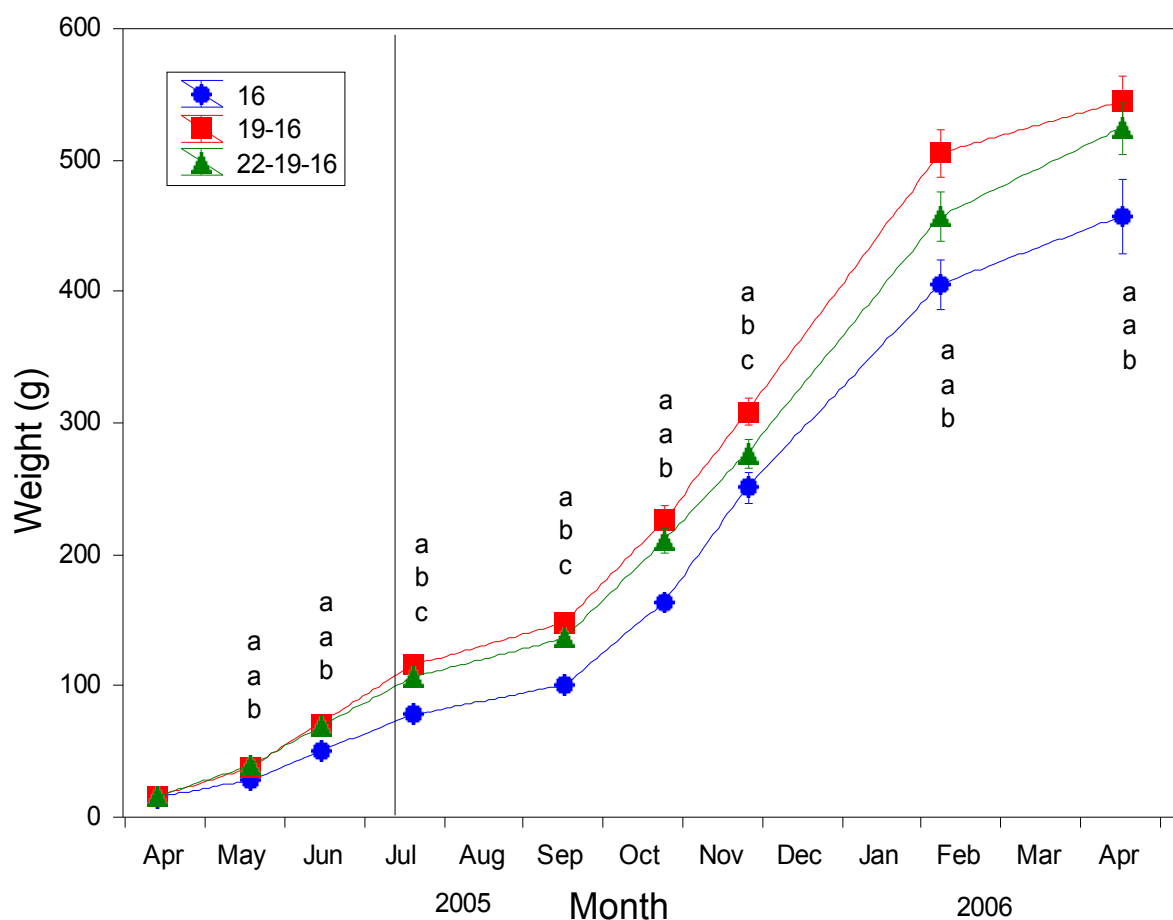


Fig. 2. Mean \pm SE weights (g) of individually tagged turbot throughout the trial period. Different letters indicate significant differences (Student-Newman-Keuls test, $P < 0.05$) between treatments in each period. The black line indicates transfer from three different temperatures to one common rearing temperature.

Mean individual growth trajectories were different (GCM, $\text{MANOVA}_{\text{TEMPERATURE}}$, Wilk's lambda (Λ)_{12, 350} = 0.28, $P < 0.001$, Fig. 3) between the three temperature groups throughout the study period. Significant differences were also found in growth-at-period trajectories of the experimental groups ($\text{MANOVA}_{\text{TEMPERATURE} \times \text{PERIOD}}$, Wilk's Λ _{10, 350} = 0.15, $P < 0.01$, Fig. 3) as growth declined between periods in all groups. Overall, the growth rate was significantly different between treatments in five out of eight experimental periods (ANOVA, $P < 0.05$, Fig. 3). In the first period the growth rate was significantly higher in the two temperature-step groups compared to the 16 group (Student-Newman-Keuls test, $P < 0.05$). The trend was reversed in the second period (days 36-62), whereas in the third rearing period the 19-16 group had a significantly higher growth rate compared to the 16 group.

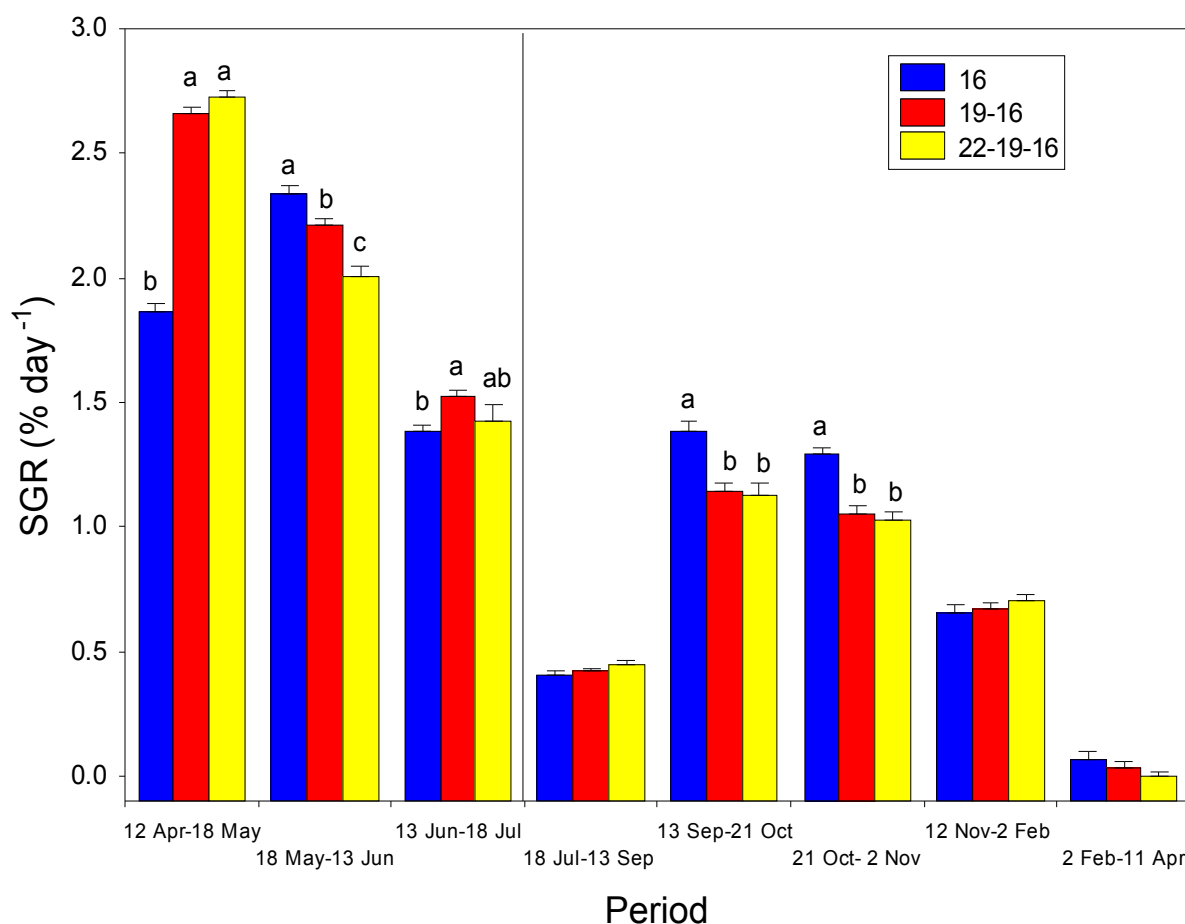


Fig. 3. Mean \pm SE specific growth rate (SGR) of individual tagged turbot during the trial. Different letters indicate significant differences (Student-Newman-Keuls test, $P < 0.05$) between treatments in each period. The black line indicates transfer from three different temperatures to one common rearing temperature.

3.2. Feeding rate and feed conversion efficiency

In the first part of the trial when the groups were reared at different temperatures the feed conversion efficiency (FCE) was higher in the 19-16 group, compared to the two other groups (One way ANOVA, $P < 0.05$, Table 1). Overall the FCE was 14-20% higher in the 19-16 group in this period (i.e. April-September 2005) reflecting the overall differences seen in mean weight for the three experimental groups (see Fig. 2).

Table 1. Feed conversion efficiency (FCE) for the three experimental groups when reared at different temperatures (April – July 2005) and when reared together (July 2005 – April 2006) at one temperature. NA=data not available.

Period	Experimental groups (separate temperatures)			All groups (one temperature)
	16	19-16	22-19-16	
April 2005 – May 2005	1.14	1.50	1.33	
May 2005 – June 2005	1.27	1.68	1.17	
June 2005 – July 2005	1.35	1.49	1.54	
July 2005 – Sept 2005				NA
Sept 2005 – Nov 2005				1.42
Nov 2005 – Feb 2006				0.92
Feb 2006 – April 2006				NA

3.3 Blood physiology

The temperature rearing conditions had only a minor effect on measured blood parameters (Table 2). In July 2005 blood pH was significantly lower and partial pressure of CO₂ (PCO_2) significantly higher in the 19-16 group (Two-way ANCOVA, $P < 0.05$). Notably plasma glucose was lowest in the 19-16 group, followed by the 22-19-16 group and highest in the 16 group at both measurement dates ($P < 0.05$).

Table 2. Measured and calculated blood parameters in juvenile turbot reared at three different temperature regimes and later at one common temperature regime. Values are given as mean (SD). Different letters denote significant differences (Student-Newman-Keuls multiple comparisons, $P < 0.05$) between treatments.

	July 2005			April 2006		
	16	19-16	22-19-16	16	19-16	22-19-16
Na ⁺ (mmol l ⁻¹)	150.14 (1.20)	153.00 (1.09)	149.86 (1.49)	154.20 (1.53)	155.62 (0.78)	154.57 (1.56)
K ⁺ (mmol l ⁻¹)	3.84 (0.20)	4.20 (0.20)	3.89 (0.20)	3.48 (0.13)	3.55 (0.12)	3.76 (0.27)
Glucose (mmol l ⁻¹)	2.33 (0.14) ^a	1.89 (0.07) ^b	2.18 (0.13) ^{ab}	3.53 (0.23) ^a	2.87 (0.18) ^b	3.22 (0.11) ^{ab}
Haematocrit (%)	13.00 (0.87)	15.60 (1.69)	13.00 (0.89)	22.00 (1.76)	21.31 (0.64)	21.86 (0.99)
pH	7.42 (0.03) ^{ab}	7.38 (0.01) ^b	7.49 (0.04) ^a	7.58 (0.03)	7.57 (0.03)	7.54 (0.05)
pCO ₂ (mmHg)	13.77 (0.29) ^b	15.92 (0.83) ^a	12.88 (0.50) ^b	7.15 (0.28)	7.58 (0.32)	7.69 (0.31)
TCO ₂ (mmol l ⁻¹)	9.03 (0.44)	9.42 (0.28)	9.86 (0.61)	6.78 (0.53)	7.05 (0.41)	6.70 (0.49)
HCO ₃ ⁻ (mmol l ⁻¹)	8.98 (0.44)	9.36 (0.28)	9.81 (0.61)	6.75 (0.53)	7.02 (0.41)	6.67 (0.49)
Haemoglobin (g dl ⁻¹)	4.41 (0.29)	5.30 (0.56)	4.44 (0.31)	7.50 (0.59)	7.25 (0.22)	7.27 (0.30)

4. PART 1. Discussion

Our study indicates that even at near-optimal temperature for a given size (*cf.* Imsland et al., 1996, 2001) the thermal history of the fish may influence growth potential, so that a short rearing period at high temperature may give a long-term growth advantage in colder water. Such long-term advantage of short-term heating was found for Atlantic cod *Gadus morhua* (Imsland et al., 2005). In that study, juvenile Atlantic cod were reared at 7, 10, 13 and 16°C, and a group reared under temperature steps (T-step) i.e. with temperature reduced successively from 16 to 13 and 10°C. The groups performed differently both during the laboratory trial and when reared at ambient conditions in the sea as the T-step group was 11.6, 11.5, 5.3 and 7.5 % larger than 7°C, 10°C, 13°C and 16°C, respectively in June 2005. Combined with the current data, these findings are important for commercial rearing of turbot as prior growth advantages can be utilized at later stages of the production. In other demersal fish species the positive effect of $T_{opt}G$ rearing scheme has been noted (e.g. spotted wolffish *Anarhichas minor*, Hansen and Falk-Petersen, 2002, Atlantic halibut *Hippoglossus hippoglossus*, Jonassen et al., 1999). In Atlantic halibut (size range, 160-400 g) reared at constant (11°C and 14°C) or switched (14°C moved to 11°C and *vice versa*) temperature regimes, Aune et al. (1997) found that growth rate was highest in fish transferred from 14°C to 11°C. This coincides with the $T_{opt}G$ for Atlantic halibut which has been shown to decrease from 14.9 to 12.7°C in the early juvenile stage (Jonassen et al., 1999). Our findings are in line with these results, demonstrating the possible gain of rearing fish at step-wise regimes instead of constant temperatures.

The changes that occur during thermal acclimation involve a series of adaptations at the enzymatic level that may lead to higher feed efficiency. There is some evidence that downward thermal acclimation (as applied in the present study) may result in increased activities in enzymes involved in aerobic energy liberation and ion transport in muscle (Jobling, 1994) and increased digestive enzyme activity (Kuzmina et al., 2003). Optimal temperature for enzymatic activity can vary with fish size (Luszkovich and Stellwag, 1993). Accordingly, the fish in the T-step groups in the present trial may have been reared closer to optimal temperatures for enzymatic than the fish in the constant temperature group. Imsland et al. (2005) showed that feed conversion efficiency (FCE) in Atlantic cod was improved in line with reduced temperature in a T-step group as FCE increased from 1.0 to 1.2 to 1.35 when cod were reared at 16°C, 13°C and 10°C, respectively. Similarly, in the present trial, FCE increased from 1.17 to 1.54 when juvenile turbot was transferred from 22 to 19°C.

Growth and plasma glucose levels were inversely correlated in our study. It has been postulated that glucose might be involved in growth control through regulation of the growth hormone/insulin-like-growth-factor-I (the GH/IGF-I system, Connors et al., 1978; Gabillard et al., 2005). Gabillard et al. (2005) found a negative correlation between glucose and GH levels which might indicate partial inhibition of GH secretion by glucose, and thereby effecting growth through the GH/IGF-I axis. Our data fits this model as the group displaying the highest growth (19-16 group) had the lowest glucose levels whereas the group displaying the lowest growth (16 group) had the highest glucose level.

Concluding remarks and increased value of sea products

In conclusion, juvenile turbot transferred from a high (22 and 19°C) to a low (16°C) temperature displayed higher growth than fish reared at a constant (16°C) temperature regime. This indicates that even at near-optimal temperature for a given size the temperature history of the fish may influence future growth. From a practical viewpoint, our results demonstrate the production advantage of rearing the fish at elevated temperatures during the juvenile period, as size differences established at this stage are maintained in the adult fish.

Although these findings are not patentable they are of considerable importance for land-based rearing of turbot in Iceland. Using these findings the farmer can maximize the growth of turbot during a production period where all turbot farmers rear their fish on land. This is, therefore, a competitive advantage compared to farmers in Spain and France, which do not have the same possibility to control rearing temperatures as Icelandic turbot farmers (utilizing geothermal heat). These findings can therefore clearly increase the production capacity, and ultimately the profitability, of Icelandic fish farming in line with the main objective of the AVS fund. It is suggested that these findings should be validated for other farmed fish species in Iceland (e.g. halibut, cod, Arctic charr), as the biological mechanism utilized in this study (i.e. size dependency of $ToptGrowth$) has been shown to be a general phenomenon in fishes (*cf.* Jonassen et al., 1999; Imsland et al., 2005).

5. Part 1. Acknowledgements

This study was financed by the European Commission (Contract: 508070 TURPRO) and by the Icelandic AVS fund (R032-05).

6. PART 1. References

- Aneer, G., Westin, L., 1990. Migration of turbot (*Psetta maxima* L.) in the northern Baltic proper. *Fish. Res.* 9, 307-315.
- Aune, A., Imsland, A.K., Pittman, K., 1997. Growth of juvenile halibut (*Hippoglossus hippoglossus* L.) under a constant and switched temperature regime. *Aquac. Res.* 28, 931-939.
- Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Sévère, A., Boeuf, F., 1996. Effects of temperature on growth and metabolism in juvenile turbot. *J. Fish Biol.* 49, 678-692.
- Connors, T.J., Schneider, M.J., Genoway, R.G., Barraclough, S.A., 1978. Effect of acclimation temperature on plasma levels of glucose and lactate in rainbow trout, *Salmo gairdneri*. *J. Exp. Zool.* 206, 443-449.
- Déniel, C., 1990. Comparative study of growth of flatfishes on the west coast of Brittany. *J. Fish Biol.* 37, 149-166.
- Gabillard, J.C., Weil, C., Rescan, P.Y., Navarro, I., Gutiérrez, J. Le Bail, P.Y., 2005. Does the GH/IGF system mediate the effect of water temperature on fish growth? A review. *Cybio* 29, 107-117.
- Hansen, T.K., Falk-Petersen, I.B., 2002. Growth and survival of first-feeding spotted wolffish (*Anarhichas minor* Olafsen) at various temperature regimes. *Aquac. Res.* 33, 1119-1127.
- Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. *Rapp. P.-V. Réun. Cons. Int. Explor. Mer.* 178, 441-453.
- Iglesias, J., Rodríguez-Ojea, G., 1994. Fitness of hatchery-reared turbot, *Scophthalmus maximus* L., for survival in the sea: first year results on feeding, growth and distribution. *Aquac. Fish. Managem.* 25 (Suppl. 1), 179-188.
- Imsland, A.K., Jonassen, T.M., 2002. Regulation of growth in turbot (*Scophthalmus maximus* Rafinesque) and Atlantic halibut (*Hippoglossus hippoglossus* L.): aspects of environment × genotype interactions. *Rev. Fish Biol. Fish.* 11, 71-90.
- Imsland, A.K., Sunde, L.M., Folkvord, A., Stefansson, S.O., 1996. The interaction between temperature and size on growth of juvenile turbot (*Scophthalmus maximus* Rafinesque). *J. Fish Biol.* 49, 926-940.
- Imsland, A.K., Folkvord, A., Grung, G.L., Stefansson, S.O., Taranger, G.L., 1997. Sexual dimorphism in growth and maturation of turbot, *Scophthalmus maximus* (Rafinesque 1810). *Aquac. Res.* 28, 101-114.
- Imsland, A.K., Foss, A., Stefansson, S.O., Nævdal, G., 2000. Hemoglobin genotypes of turbot (*Scophthalmus maximus*): variation in growth and optimal temperature for growth. *Fish Physiol. Biochem.* 23, 75-61.
- Imsland, A.K., Foss, A., Gunnarsson, S., Berntssen, M., FitzGerald, R., Bonga, S.W., van Ham, E., Nævdal, G., Stefansson, S.O., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 198, 353-367.
- Imsland, A.K., Foss, A., Folkvord, A., Stefansson, S.O., Jonassen, T.M., 2005. The interrelation between temperature regimes and fish size in juvenile Atlantic cod (*Gadus morhua*): effects on growth and feed conversion efficiency. *Fish Physiol. Biochem.* 31, 347-361.
- Imsland, A.K., Wergeland, T., Jonassen, T., Stefansson, S.O. 2006. Does malpigmentation improve growth in juvenile turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*). *Aquac. Res.* 37, 306-312.
- Irwin, S., O'Halloran, J. and FitzGerald, R.D. 1999. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). *Aquaculture* 178, 77-88.

- Iwama, G., 1998. Stress in fish. *Ann. New York Acad. Sci.* 851, 304-310.
- Jonassen, T.M., Imslund, A.K., Stefansson, S.O., 1999. The interaction of temperature and size on growth of juvenile Atlantic halibut. *J. Fish Biol.* 54, 556-572.
- Kuzmina, V., Glatman, L., Drabkin, V., Gelman, V., 2003. Amylolytic activity in fish intestinal mucosa: temperature effects. *Comp. Biochem. Physiol. B – Biochem. Molec. Biol.* 134, 529-534.
- Luczkovich, J.J., Stellwag, E.J., 1993. Isolation of cellulotic microbes from the intestinal-tract of the pinfish, *Lagodon rhomboides*, size-related changes in diet and microbial abundance. *Mar. Biol.* 116, 381-388.
- Timm, N.H., 1980. Multivariate analysis of variance of repeated measurements. In: Krishnaiah, P.R., (Ed.), *Handbook of Statistics, Vol. 1, Analysis of Variance.* North-Holland Publishing Company, Amsterdam, Netherlands, pp. 41-87.
- Zar, J.H., 1984. *Biostatistical Analysis*, 2nd edition, Prentice-Hall, Englewood Cliffs, 718 pp.

PART 2 – Slaughter quality and rigor contraction in farmed turbot: a comparison between different stunning methods.

Summary R 032 05 – Part 2

To evaluate the importance of different stunning and killing methods on the flesh quality, a total of 80 market sized farmed turbot were taken from its holding tanks at Silfurstjarnan, Iceland and slaughtered in 4 different ways. Groups of 20 fish were either killed directly by a percussive blow to the head and: 1) stored untreated on ice, 2) electrical stimulated with 5 Hz pulsed direct current (pDC); 3) electrical stimulated with 80 Hz pDC; or 4) commercially killed by live exsanguination into bins of ice slurry for 1 h. All fish were stored in boxes with ice. Muscles pH, *rigor mortis*, texture hardness were measured over a 7 day time span. Results show that fish exsanguinated live or electrical stimulated exhibited a rapid pH decline and earlier onset and resolution of *rigor mortis*. After 7 days of storage no significant differences could be detected in texture attributed measured as shear force or hardness between the groups of fish. Furthermore *pre mortem* muscle activity associated with the anaerobe muscle activity has little or no impact on the end quality of turbot. From these grounds it appears to be safe to expose turbot for longer periods of electricity to secure permanent insensibility without destroying the quality.

Skýrsluágríp R 032 05 – Hluti 2

Markmið rannsóknar var að kanna áhrif mismunandi aflífgunaraðferða á holdgæði eldissandhverfu. Alls voru 80 einstaklingsmerktir fiskar teknir úr eldiskerjum á Silfurstjörnum og slátrað með ferns konar hætti. Hópar með 20 fiskum voru drepnir á eftirfarandi hátt; gefið rothögg við háfun úr kerri og 1) geymdir ómeðhöndlaðir á ís, 2) gefið rafstuð með 5 Hz styrkleika (pDC), 3) gefið rafstuð með 80 Hz styrkleika (pDC) eða 4) blóðgaðir og látnir liggja í ískrapa í 1 klst. (hefðbundin slátrunaraðferð). Eftir meðhöndlun voru allir fiskar geymdir á ís í frauðplastskössum. Á sjö daga tímabili var fylgst með pH gildi í holdi, dauðastirðnun og áferð/hörku. Niðurstöður sýna að hjá fiski sem blóðgaður var lifandi eða gefið rafstuð féll sýrustig í holdi hraðar og dauðastirðnun gekk fyrir yfir. Á sjöunda degi frá slátrun var enginn munur á milli hópa hvað snertir áferð holds (skerkraftsmæling eða harka). Að auki virðist súrefnissnaður bruni í vöðvum sem afleiða af streitu og álagi fyrir slátrun hafa lítil eða engin áhrif á holdgæði hjá sandhverfu.

PART 2 – Slaughter quality and rigor contraction in farmed turbot: a comparison between different stunning methods

1. Part 2. Introduction

In Europe the most common stunning and killing method for farmed turbot (*Scophthalmus maximus*) is a thermal insult in combination with exsanguination. How this affects the welfare and quality is not well documented enough for any conclusions, although it is generally assumed to be detrimental to good welfare (Robb and Kestin 2002; van de Vis et al. 2003). Stress associated with stunning and slaughter of is commonly known to affect flesh quality by an early onset on *rigor mortis*, softening of the texture, gaping, drip loss and loss of shelf life (Boggess et al. 1973; Kiessling et al. 2004; Marx et al. 1997; Roth et al. 2006a). As an alternative to a thermal insult, electricity has proven to be a fast and efficient method to both stun and/ or kill fish (Lambooij et al. 2006; Lines et al. 2003; van de Vis et al. 2003). For turbot, a two stage stun is likely the optimum way for stunning turbot, by first exposing the animal for AC currents for a few seconds to stun the animal unconscious; and secondly maintaining a low frequency current for a few minutes to secure either permanent or longer periods of insensibility.

It is known that an electric shock can cause injuries as the muscles contract, causing dislocation or a fracture of the spinal column, ripping off the dorsal aorta or veins, leading to hematomas in the fillet. The muscle contraction strength depends on current type, field strength and frequency (Lines and Kestin 2005; Roth et al. 2004). Hence, salmonids appear to be most sensible for electric currents in the region of 50-100 Hz AC. On turbot no electric related injuries has been reported (Morzel et al. 2003; Ruff et al. 2002), although these aforementioned reports lack enough details to be conclusive.

Electrical stimulation is known to affect the quality of mammalian meat whereas softening of meat is closely related to the energy metabolism (Fabiansson and Buchter 1984; Fabiansson and Reutersward 1985; Maribo et al. 1999). There are uncertainties on the underlying mechanisms and factors which contribute to the *post mortem* softening of the flesh in fish, but previous reports by Roth et al. (2006a) shows that electrically

exercised Atlantic salmon muscles using 5 Hz pDC exhibited a much faster onset of *rigor mortis* than i.e. stressed and unstressed fish, but the texture traits from electrically stimulated carcasses were similar to what was observed in unstressed fish. Similar Morzel et al. (2003) reported an earlier onset of *rigor mortis* and slight loss of muscle shear force in electrically stimulated turbot, but found no evidence of structural degradation, water holding capacity or changes of sensory attributes.

The aim of this study was to evaluate how stress and muscle metabolism affects the quality of turbot fillets. Comparing texture properties from rested, stressed fish and electrical stimulated fish using either 5 or 80 Hz AC could provide answers on how various slaughter methods affect the end quality in farmed turbot.

2. Part 2. Material and Methods

2.1 General

In order to identify what effect slaughtering methods and muscle activity had on the quality of farmed turbot, a total of 80 turbot with mean weight equal to $1.2 \text{ kg} \pm 0.17 \text{ SD}$ (Table 3) were on day 1, the 22 November 2005, slaughtered in 4 different ways at Silfurstjarnan, Iceland. The fish were either killed directly by a percussive blow to the head and: 1) stored untreated (unstressed), 2) electrical stimulated with 5 Hz pulsed direct current (pDC) (el-stim 5 Hz), 3) electrical stimulated with 80 Hz pDC (el-stim 80 Hz) or 4) live exsanguinated in bins of ice slurry (exsanguinated). From all experimental groups, half of the fish (n=40) were used to follow muscle pH and *rigor mortis* over a 4 day period, while the other half of fish (n=40) were stored for 6 days before measuring shear force and hardness. All fish were stored on boxes on ice and internal temperature was monitored for 48 h.

Table 3. Average round weight (kg) \pm SE of turbot used in experiments for muscle pH, rigor and texture. In each column different capital letters represent a significant difference of $P < 0.05$. $N = 80$.

	Texture analysis		pH and rigor	
	Mean	SE	Mean	SE
Unstressed	1.25 ^a	0.048	1.30 ^a	0.062
Exsanguinated	1.16 ^{a,b}	0.052	1.28 ^a	0.056
El-stim 5 Hz	1.08 ^{b*}	0.046	1.23 ^a	0.057
El-stim 80 Hz	1.18 ^{a,b}	0.035	1.17 ^a	0.045

2.2 Experimental protocol

For electrical stimulation a pulse generator was used made by ARENA A/S (inc). The apparatus provided a pure squared direct current (pDC), max 26 Volts (V), max 5 amps (A) with frequencies (f) between 1-80 Hz. The duty cycle of the square pulses was set to 50 % providing a pulse length (ms) equal to $1000/(2*f)$ ms. The potential difference was measured in root mean square, while peak values were exactly twice as high as root mean square values.

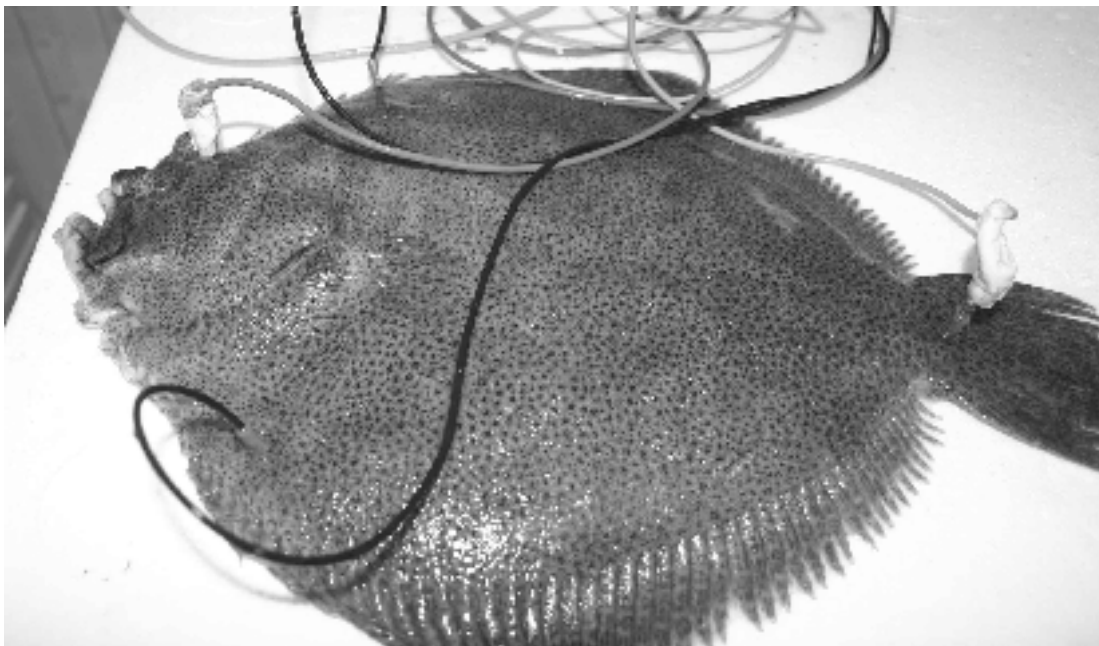


Fig. 4. Electrical stimulation of turbot. Anode is placed in the head and at both sides at the frontal part of the muscle, while the cathode was placed into the caudal region.

The electrical stimulation was carried out on carcasses by placing the anode into the

neurocranium and the cathode into the vertebrae by the tail (Fig. 4). Two electrodes (anodes) were placed on the outer parts of the fillets to ensure a more homogenous electric field (Fig. 4). The electric stimulation started approximately 5 min *post mortem* and the duration of the electrical stimulus was 3 min. For exsanguination fish were slaughtered as commercially practiced; where the turbot was first placed into bins of ice slurry for approximately 30 min before they were exsanguinated and again placed into bins of ice slurry and left to bleed for approximately 1 h before gutting.

Muscle pH

For measuring muscle pH, an X-Mate portable meter and Inlab 489 pH probe from Mettler Toledo™ was used. Muscle pH was obtained from the white muscle tissue along the loins on both dorsal sides where the lateral lines curves. Muscle pH was never measured in wounds from previous pH sampling. Muscle pH was measured at 0, 3, 6, 12, 24, 48 and 72 h *post mortem*. Muscle pH was also measured on day 7 in all fish used for texture analysis.

Rigor mortis

The rigor index (I_r) was obtained using cuttings method (tail drop). The rigor index was calculated by following formula $I_r = [(L_0 - L_t) / L_0] \cdot 100$ (Bito et al. 1983). L represents the vertical drop (cm) of the tail, when half of the fish fork length is placed on the edge of a table. L_0 is the tail drop at the beginning of the experiment, while L_t represents measurements throughout the experiment. Rigor was measured at 0, 3, 6, 12, 24, 48 and 72 h *post mortem*. The rigor index was also measured on day 7 using the other half of fish used for texture analysis.

Texture shear force and hardness

For texture measurements both fillets from the dorsal back were filleted off the fish and used for measuring the Kramer shear force (blade) and hardness (puncture), separately using a TA-XT2® Texture Analyzer from Stable Micro Systems with a load cell of 25 kg. For shear force measurements, a 3 × 70 mm blade with 60 ° knife edge, was used to slice standard muscle samples (69 × 26 mm) with constant speed at 0.8 mm/s (Sigurgisladottir

et al. 2001). Shear force was measured at 2 locations on each muscle sample providing a total of 4 samples pr. fish.

Texture hardness was done according to Ruff et al. (2002) using a 20 mm of diameter flat cylinder test probe. The penetration depth for the probe was 3 mm holding a constant speed equal of 0.8 mm/s (Ruff et al. 2002). The texture profile was sampled at 4 locations directly on the dorsal fillet from the downwards side of the fish using a fixed distance (30 mm).

2.3 Statistical analysis

The statistical analysis was performed on Statistica 7.1. For analysis of muscle pH as a function of both categorical (treatment) and continuous (time), variables covariate analysis (ANCOVA) was used as the statistical model.

To reveal an interaction between hardness and fillet thickness correlation analysis (Standard Pearson) was used and the fillet thickness was incorporated as a covariate into factorial ANCOVA on hardness. ANCOVA was used for testing independent and continuous variables as shear force and hardness against categorical variables such as treatment and fillet location, where fillet thickness was incorporated as an covariate. For post hoc analysis Scheffels test were used. The level of significance was set $P < 0.05$.

3. Part 2. Results

3.1 General

Upon commercial slaughter, the fish did respond to entry into the ice-bin by quickly swimming a couple of laps around the bin before they settled on the bottom. Upon exsanguination, half an hour later, the average muscle pH was 7.02 ± 0.08 (SD) and the fish were still physically active. One hour after exsanguination most of the fish was still active and had to be manually killed by a percussive stroke to the head for further analysis. Also it was difficult to kill the fish with percussive stunning. One percussive stroke to the head was not always enough, so multiple hammer strokes had to be applied in order to be certain that the fish was dead. No external or internal injuries could be observed among the fish that were electrical stimulated, regardless of electric frequency.

We did, however, observe that fish exposed for a more stressed environment prior to death excreted more black mucus than its unstressed counterparts during measurements on rigor. For the fish stored untreated for 6 days before texture analysis, no difference in mucus could be detected. Fillet gaping was completely absent in turbot fillets.

3.2 Rigor mortis

As shown in Figure 5, fish exsanguinated alive or exposed to 3 min of electricity exhibit a faster onset and resolution of *rigor mortis* than the unstressed fish. No significant differences in stages of rigor amongst exsanguinated and electrical stimulated fish was seen (Fig. 5).

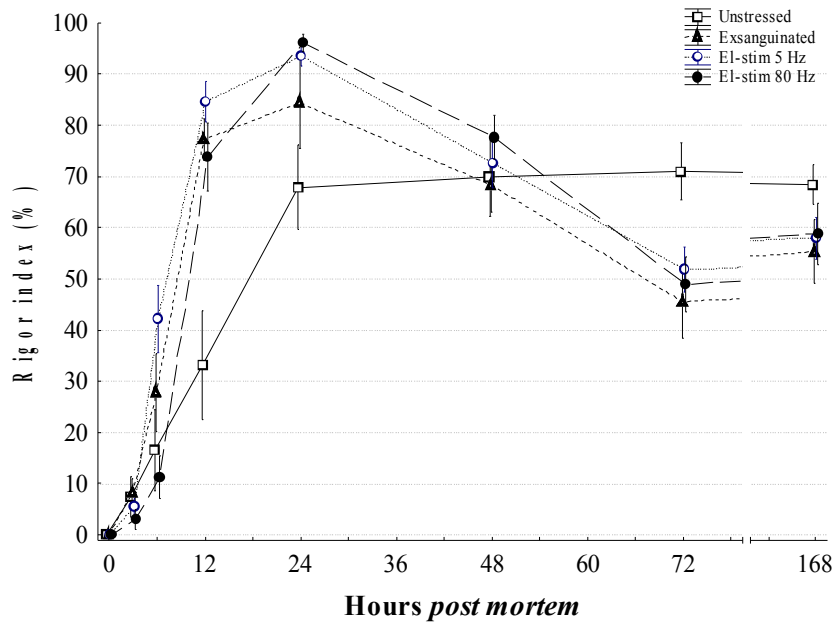


Fig. 5. Mean rigor index \pm SE for fish that was percussive killed and 1) stored untreated (unstressed); 2) electrical stimulated with 5 Hz pulsed direct current pDC (el-stim 5 Hz); 3) electrical stimulated with 80 Hz pDC (el-stim 80 Hz) or 4) exsanguinated live and placed in bins of ice slurry (exsanguinated). N=10 for each group

All of the treated fish had maximum rigor within 24 h *post mortem*, while within unstressed fish, 30 % of the fish had maximum rigor tensions within 24 h *post mortem*, and 90 % within 48 h.

3.3 Muscle pH

The anaerobic muscle activity caused by live exsanguination and electrical stimulation caused a significant drop of muscle pH at time of death ($P < 0.005$, ANCOVA). As shown in Figure 6, the muscle pH in fish exsanguinated alive was similar to what was observed in electrical stimulated fish. The pH drop in exsanguinated fish is more likely due to anaerobe metabolism from elimination of the circulatory flow rather than the intense muscle activity as observed in electrical stimulated fish. The muscle pH in treated fish increased after 48 h of storage, at a time when the fish was resulting from *rigor mortis*.

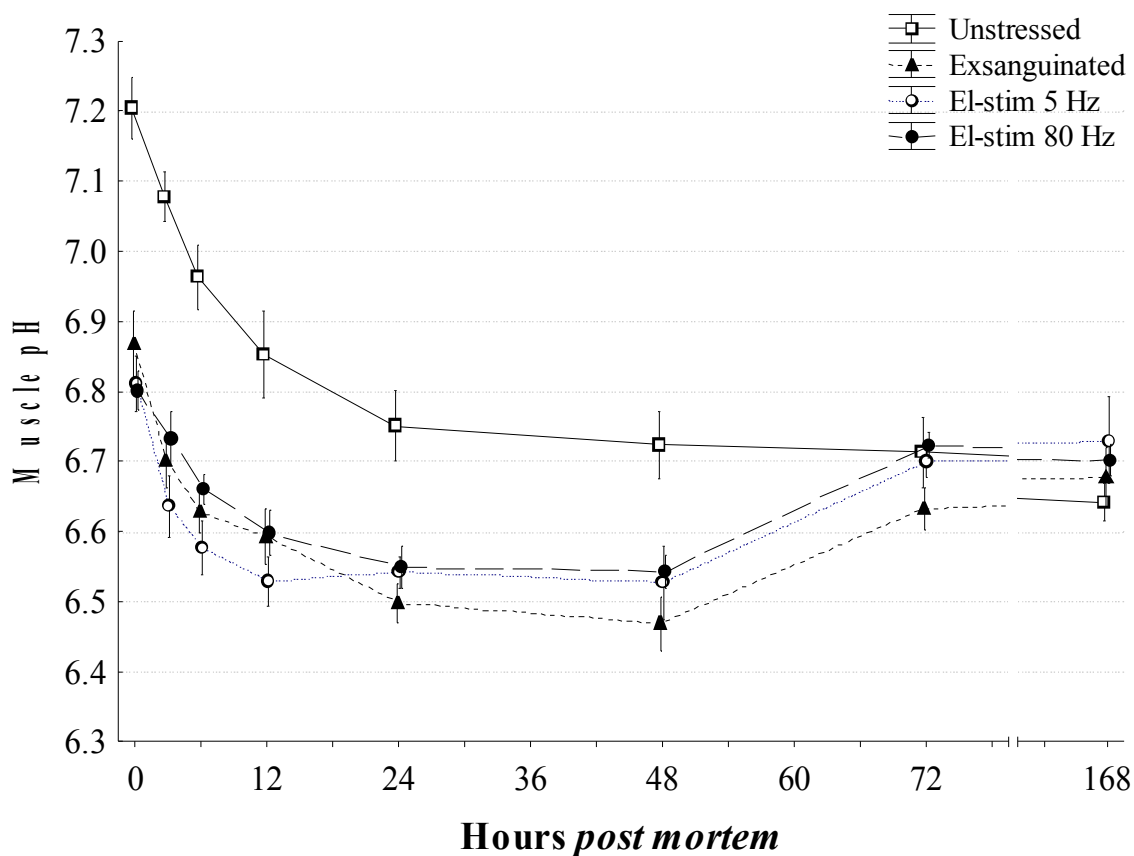


Fig. 6. Average muscle pH \pm SE in fish that was percussive killed and 1) stored untreated (unstressed); 2) electrical stimulated with 5 Hz pulsed direct current pDC (el-stim 5 Hz); 3) electrical stimulated with 80 Hz pDC (el-stim 80 Hz) or 4) exsanguinated live and placed in bins of ice slurry (exsanguinated). N=10 for each group.

At 6 days *post mortem* there were no significant differences in muscle pH between the groups, although the ranking of end pH was transverse of the ranking pH at the beginning.

3.4 Texture hardness and shear force

Results on shear force (Table 4) show no significant difference between the different treatment groups ($P>0.26$; factorial ANOVA). There were however significant differences in shear force according to the location on the fillet ($P<0.001$, factorial ANOVA), as location 2 and 3 had lower shear force values than 1 and 4. No interaction was observed between location of the fillet and treatment groups ($P > 0.68$, factorial ANOVA). There were no correlation between fillet thickness and shear force ($R=0.1$, $P>0.205$, $N=155$) explaining that the variation was not caused thickness.

Using the puncture test, show that the hardness was closely related to the percentage of compression, which decreased with fillet thickness ($R=0.68$, $P<0.0001$, correlation analysis). Incorporating compression as a covariate into factorial ANOVA revealed that location of the fillet was less of significance ($P>0.113$; ANCOVA), while there was a significant difference in treatment ($P<0.05$; ANCOVA). Post hoc analysis showed that exsanguinated fish had a significant harder texture than both electrical stimulated carcasses using 5 Hz pDC and unstressed fish ($P<0.05$).

Table 4. Muscle shear force and hardness (N) in percussive killed and 1) stored untreated (unstressed); 2) electrical stimulated with 5 Hz pulsed direct current pDC (el-stim 5 Hz); 3) electrical stimulated with 80 Hz pDC (el-stim 80 Hz) or 4) exsanguinated live and placed in bins of ice slurry (exsanguinated). Muscle samples were taken at 4 different locations on the dorsal fillet. Results are given in least square mean and different capital letters represents a significant difference of $P < 0.05$.

	Group	Average force on whole fillet (N)		Force (N) from location on the fillet (1-4)								N
		mean	SE	1		2		3		4		
Shear force (Blade)	Unstressed	98.1 ^a	3.93	98.1	7.76	93.0	7.76	93.3	7.76	108.0	8.18	10
	El-stim 5 Hz	92.4 ^a	3.88	96.2	7.76	89.6	7.76	85.3	7.76	98.4	7.76	10
	El-stim 80 Hz	94.6 ^a	3.99	98.6	7.76	83.4	7.76	98.4	8.18	98.2	8.18	10
	Exsanguinated	100.5 ^a	3.93	115.1	7.76	96.7	7.76	90.2	8.18	100.2	7.76	10
	All groups			102.0 ^a	3.88	90.7 ^b	3.88	91.8 ^b	3.99	101.2 ^a	3.99	
Hardness (Puncture)	Unstressed	15.8 ^a	1.38	13.7	3.11	16.2	2.74	19.7	2.54	13.4	2.62	5

4. Part 2. Discussion

In correspondence with previous studies on turbot (Morzel et al. 2003) and salmon (Roth et al. 2006b), the results on chilled and live exsanguinated fish show that the fish are expressing anaerobe metabolic activity, meaning that fish are not unconscious and are physically active during bleed-out (Fig. 3). A pH drop from 7.2 down to 6.8-6.9 resembles the muscle pH in electrically exhausted carcasses (Fig. 3). Apparently exsanguination of live fish in ice water is not an appropriate method in terms of welfare. Morzel et al. (2003) reported that live exsanguinated turbot in ice slurry were capable of expressing physically responses for at least 15-30 min, while fish exsanguinated in ambient temperatures could express behavioral responses for at least 90 min. This is in line with current experience, where many of the experimental fish had to be manually killed 1 h after exsanguination, and considering that commercial practice often involves gutting shortly after exsanguination, the method of exsanguination fish live could be regarded as inhumane.

Percussive stunning was very difficult to obtain on turbot without inflicting damage to the carcasses, where eye prolaps was often the result. For salmonids eye prolapse is often a result from manually stunning using hand held priests and not from percussive machines (Roth et al. 2007). However injuries such as eye bursts or hemorrhaging in the eye is closely related to the bolt force (Roth et al. 2007). Since the eyes in turbot are almost directly above the neurocranium, a percussive stun from above is difficult to obtain without injuring the eyes. Furthermore, applying a percussive force from the side is an alternative, but since the neurocranium and eyes can migrate either to the left or right during metamorphosis, it becomes difficult to foresee automated operations that can hit, stun and kill turbot.

Electrical stunning appears to be a fast and appropriate alternative for stunning farmed turbot (Morzel et al. 2003). In order to maximize the chances for achieving permanent insensibility a two stage stun is likely the best alternative. Applying a prolonged electric exposure after the first stun will can contribute to death or postpone the unconscious condition into death by exsanguination. However the prolonged electric exposure may be of concern for the end quality as anaerobe glycolysis associated with stress is known to affect the quality in fish as Atlantic salmon (Kiessling et al. 2004). In line with the results on pH, the live exsanguinated fish and the electrical stimulated carcasses exhibited an early onset and resolution of *rigor mortis* (Fig. 2). However no significant differences could be verified between texture traits between the stressed, exercised and unstressed group (Table 2). The reason is unclear. Previous studies on turbot shows that stress does lead to softer texture

measured as hardness (Ruff et al. 2002) and shear force (Morzel et al. 2003). Measuring hardness according to Ruff et al. (2002) using a fixed distance for compressing the fish meat at different locations is unreliable as the hardness is dependent on the fillet location and thereby thickness of the fillet. However the conditions in our experiment do resemble the experimental conditions carried out by Morzel et al. (2003). The discrepancy in shear force results is not surprising. Morzel et al. (2003) did report significant lower shear forces in electrically stunned turbot as compared to live exsanguinated and percussive stunned fish over a period of 9 days *post mortem*. However, the textural differences between the slaughter groups remained more or less unchanged already from on first measurement on day 0 suggesting that individual differences is a more likely cause of differences in shear force rather than stunning methods. Similar to mammalian meat, the flesh quality in fish seems to be related to stress (Kiessling et al. 2004; Marx et al. 1997; Roth et al. 2002), but contradictory studies does also exist showing that anaerobe glycol sis does not lead to a softer texture (Jittinandana et al. 2005; Roth et al. 2006a; Scherer et al. 2006; Stien et al. 2005). The reason for this discrepancy is unclear. One possible explanation for the stress related acceleration in softening of the flesh could be excessive muscle bursts associated with flight reactions (Roth et al. 2006a). It has been shown that that intense exercise prior to death causes a higher proteolysis during storage (Belcastro et al. 1998; Morzel et al. 2006). It is however unclear whether the increased proteolysis is directly related to the muscle pH, violent muscle bursts or both?

The use of electricity to stimulate carcasses and simulate anaerobic metabolism is an interesting tool for understanding what mechanisms causes softening of the flesh. It is well known that electrical stimulation of muscles causes a rapid depletion of high energy phosphogenes like ATP and PCr (Chiba et al. 1990) and that exposure for longer periods of electricity causes a pH drop and rapid onset of *rigor mortis* (Fletcher et al. 1997; Jerrett et al. 1996; Jerrett and Holland 1998). How the muscles response to the applied current strength and frequency and the subsequent effect on quality is not well studied. Frequencies in the region of 50 to 100 Hz leads to the strong muscle contractions maximizing the animals risk for spinal injuries, but are also optimum frequencies for stimulating the CNS and thereby stun the animal (Lines et al. 2003; Roth et al. 2004). The fact that turbot was not injured using 80 Hz pDC is indeed promising as hematomas associated with spinal injuries is a major quality problem for other farmed species such as Atlantic salmon.

From this ground it is reason to expect that animal exposed to 80 Hz pDC would have softer flesh than fish exposed for 5 Hz pDC. As shown in Figure 2, 3 and Table 2, the

carcasses exposed for 80 Hz did not differ in any quality traits compared to the other treated groups. From the electrical stimulation trials it is clear that the muscles response to electricity is vague as compared to Atlantic salmon. Apparently spinal injuries and hemorrhaging have never been observed in electrically stunned turbot and considering that the quality is hardly affected indicates that a two stage stun can easily be adopted for turbot with success.

Concluding remarks and increased value of sea products

We conclude that stress through live exsanguination and prolonged electrical stimulation does cause a rapid drop of muscle pH and onset of *rigor mortis* in turbot, but the textural traits as hardness and shear force remained unchanged. We therefore conclude that the prolonged electrical exposure associated with a two stage stun used to enhance welfare issues related to stunning and killing of turbot does not influence the flesh quality in any major ways.

Results in the current study clearly show that the flesh quality in turbot is affected by stunning method. Although the findings of this study are not patentable they are of considerable practical interest for turbot farmers. Firstly, it is important to have a clear view of how different slaughter methods affect the final product. The knowledge obtained in this study will be beneficial for the company involved (Silfurstjarnan) as for the whole Icelandic farming industry, as increased knowledge on slaughter is of paramount interest for the farming industry. Overall, the methodological development employed in this project will increase the value of the product (here, slaughter size turbot) as the industry can apply slaughter methods that lead to optimal quality of the end product as well as prolonging *rigor mortis*. This is clearly in line with the main objective of the AVS fund.

It is suggested that these findings should be validated for other farmed fish species in Iceland (e.g. halibut, cod, Arctic charr), as evaluation on how stress and muscle metabolism affects the quality of farmed fish are needed to develop the industry further.

5. Part 2. Acknowledgements

These experiments was a part of the EC-CRAFT project no. 508070, TURPRO and AVS project R032-05. We would like to thank all the involved project partners A.Coelho e Castro, Portugal; Ecomares, Germany; Llyn Aquaculture Ltd, U.K; Silfurstjarnan, Iceland; and Zeeland Vis BV, Netherlands. Also thanks to Soffia Vala Tryggvadottir at the Icelandic Fisheries Laboratory for technical support during the experiment and Edward Schram at Wageningen IMARES, The Netherlands.

6. Part 2. References

- Belcastro, A. N., Shewchuk, L. D., Raj, D. A., 1998. Exercise-induced muscle injury: A calpain hypothesis. *Molecular Cell. Biochem.* 179, 135-145.
- Bito, M., Yamada, K., Mikumo, Y., Amano, K., 1983. Difference in the mode of rigor mortis among some varieties of fish by modified Cutting's method. *Bull. Tokai. Reg. Fish. Res. Lab.* 109, 89-93.
- Boggess, T. S., Heaton, E. K., Shewfelt, A. L., Parvin, D. W., 1973. Techniques for Stunning Channel Catfish and Their Effects on Product Quality. *Journal of Food Science* 38, 1190-1193.
- Erikson, U., Sigholt, T., Rustad, T., Einarsdottir, I. E., Jorgensen, L., 1999. Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. *Aquacult. Int.* 7, 101-115.
- Fabiansson, S., Buchter, L., 1984. The Influence of Low-Voltage Electrical-Stimulation on Some Physical and Sensoric Properties of Beef. *Acta Agric. Scandinavica* 34, 368-376.
- Fabiansson, S., Reutersward, A. L., 1985. Low-Voltage Electrical-Stimulation and Post-Mortem Energy-Metabolism in Beef. *Meat. Sci.* 12, 205-223.
- Fletcher, G. C., Hallett, I. C., Jerrett, A. R., Holland, A. J., 1997. Changes in the fine structure of the myocommata-muscle fibre junction related to gaping in rested and exercised muscle from king salmon (*Oncorhynchus tshawytscha*). *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* 30, 246-252.
- Jerrett, A. R., Holland, A. J., 1998. Rigor tension development in excised "rested", "partially exercised" and "exhausted" chinook salmon white muscle. *Journal of Food Science* 63, 48-52.
- Jerrett, A. R., Stevens, J., Holland, A. J., 1996. Tensile properties of white muscle in rested and exhausted chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Food Science* 61, 527-532.
- Jittinandana, S., Kenney, P. B., Mazik, P. M., Danley, M., Nelson, C. D., Kiser, R. A., Hankins, J. A., 2005. Transport and stunning affect quality of arctic char fillets. *Journal of Muscle Foods* 16, 274-288.
- Kiessling, A., Espe, M., Ruohonen, K., Morkore, T., 2004. Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO₂ anaesthesia. *Aquaculture* 236, 645-657.
- Lambooij, B., Kloosterboer, K., Gerritzen, M. A., Andre, G., Veldman, M., van de Vis, H., 2006. Electrical stunning followed by decapitation or chilling of African catfish (*Clarias gariepinus*): assessment of behavioural and neural parameters and product quality. *Aquacult. Res.* 37, 61-70.
- Lines, J., Kestin, S., 2005. Electric stunning of trout: power reduction using a two-stage stun. *Aquacultural Engineering* 32, 483-491.
- Lines, J. A., Robb, D. H., Kestin, S. C., Crook, S. C., Benson, T., 2003. Electric stunning: a humane slaughter method for trout. *Aquacult. Eng.* 28, 141-154.
- Maribo, H., Ertbjerg, P., Andersson, M., Barton-Gade, P., Moller, A. J., 1999. Electrical stimulation of pigs - effect on pH fall, meat quality and Cathepsin B+L activity. *Meat Science* 52, 179-187.
- Marx, H., Brunner, B., Weinzierl, W., Hoffmann, R., Stolle, A., 1997. Methods of stunning freshwater fish: Impact on meat quality and aspects of animal welfare. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology* 204, 282-286.
- Morzel, M., Chambon, C., Lefevre, F., Paboeuf, G., Laville, E., 2006. Modifications of trout (*Oncorhynchus mykiss*) muscle proteins by preslaughter activity. *J. Agric. Food Chem.* 54, 2997-3001.
- Morzel, M., Sohier, D., van de Vis, H., 2003. Evaluation of slaughtering methods for turbot with respect to

- animal welfare and flesh quality. *J. Sci. Food Agric.* 83, 19-28.
- Robb, D. H. F., Kestin, S. C., 2002. Methods used to kill fish: Field observations and literature reviewed. *Animal Welfare* 11, 269-282.
- Roth, B., Moeller, D., Slinde, E., 2004. Ability of electric field strength, frequency, and current duration to stun farmed Atlantic salmon and pollock and relations to observed injuries using sinusoidal and square wave alternating current. *N. Am. J. Aquacult.* 66, 208-216.
- Roth, B., Moeller, D., Veland, J. O., Imsland, A., Slinde, E., 2002. The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (*Salmo salar*). *J. Food Sci.* 67, 1462-1466.
- Roth, B., Slinde, E., Arildsen, J., 2006a. Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. *Aquaculture* 257, 504-510.
- Roth, B., Slinde, E., Robb, D. H. F., 2006b. Field evaluation of live chilling with CO₂ on stunning Atlantic salmon (*Salmo salar*) and the subsequent effect on quality. *Aquacult. Res.* 37, 799-804.
- Roth B., Slinde, E., Robb, D.H.L., 2007. Percussive stunning of Atlantic salmon (*Salmo salar*) and the relation between force and stunning. *Aquacult. Eng.* 36, 192-197.
- Ruff, N., Fitzgerald, R. D., Cross, T. F., Teurtrie, G., Kerry, J. P., 2002. Slaughtering method and dietary alpha-tocopheryl acetate supplementation affect rigor mortis and fillet shelf-life of turbot *Scophthalmus maximus* L. *Aquacult. Res.* 33, 703-714.
- Scherer, R., Augusti, P. R., Bochi, V. C., Steffens, C., Fries, L. L. M., Daniel, A. P., Kubota, E. H., Neto, J. R., Emanuelli, T., 2006. Chemical and microbiological quality of grass carp (*Ctenopharyngodon idella*) slaughtered by different methods. *Food Chemistry* 99, 136-142.
- Sigurgisladottir, S., Sigurdardottir, M. S., Ingvarsdottir, H., Torrissen, O. J., Hafsteinsson, H., 2001. Microstructure and texture of fresh and smoked Atlantic salmon, *Salmo salar* L., fillets from fish reared and slaughtered under different conditions. *Aquacult. Res.* 32, 1-10.
- Stien, L. H., Hirmas, E., Bjornevik, M., Karlsen, O., Nortvedt, R., Rora, A. M. B., Sunde, J., Kiessling, A., 2005. The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). *Aquacult. Res.* 36, 1197-1206.
- van de Vis, H., Kestin, S., Robb, D., Oehlenschlager, J., Lambooi, B., Munkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Ottera, H., Roth, B., Sorensen, N. K., Akse, L., Byrne, H., Nesvadba, P., 2003. Is humane slaughter of fish possible for industry? *Aquaculture Research* 34, 211-220.

PART 3 – Exsanguination of turbot and the effect on fillet quality measured mechanically, sensory and with computer vision

Summary R 032 05 – Part 3

The meat quality of bled and non bled farmed turbot was evaluated using mechanical, sensory, and computer imaging techniques revealing what impact the blood residues had on the end quality. Results show that exsanguination is important for improving the visual appearance, and the blood residue could be quantified using computer imaging system. After 6 days of storage, mechanical analysis using puncture test or Kramer shear force showed no difference between bled and nonbled fish. The trained taste panel were unable to detect any differences between bled and unbled fish after 6 and 13 days of storage. We conclude that in over a two week period the blood residue in turbot meat only affects the visual quality.

Skýrsluágríp R 032 05 – Hluti 3

Gæði fiskholds í blóðgaðri og óblóðgaðri eldissandhverfu voru metin með aðstoð reynsluprófa og skynfræðilegra aðferða. Auk þess var tölvusjón nýtt til að meta áhrif blóðleifa á gæði sláturfisks. Niðurstöðurnar sýna að blóðgun er mikilvæg til að bæta sjónræn útlit, og hægt var að ákvarða magn blóðleyfa með aðstoð tölvusjónar. Eftir 6 daga geymslu sýndu reynslupróf (stautun og Kramer skerkræftsmæling) engan mun á milli blóðgaðs og óblóðgaðs fisk. Í skynmatsprófi með þjálfuðum dómurum fannst ekki munur á milli blóðgaðs og óblóðgaðs fisk eftir 6 og 13 daga geymslu. Við ályktum að innan tveggja vikna frá slátrun hafi blóðleifar í sandhverfuhaldi einungis áhrif á sjónrænt útlit fisk.

PART 3 – Exsanguination of turbot and the effect on fillet quality measured mechanically, sensory and with computer vision

1. PART 3. Introduction

In Europe the procedures for exsanguination of farmed turbot (*Scophthalmus maximus*) varies according the culture and market. In northern Europe it is common to exsanguinate the fish prior to gutting, while in southern Europe the fish is often sold whole and unbled. For other farmed species such as rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) the norm is to exsanguinating all fish by cutting one or two sets of gill arches prior to gutting.

Besides having a negative appearance on the visual quality (Robb and others 2003; Roth and others 2005; Olsen and others 2006), blood residue in the flesh is a potential source for lipid oxidation. A hemin or iron release from hemo and myoglobin during hemolysis acts as a catalyst for lipids oxidation in parallel to formation of superoxide, hydrogen peroxide and hydroxyl radicals (Misra 1972; Puppo and Halliwell 1988a, b; Grunwald and Richards 2006a, b). For fish as food, residue blood has shown have a negative effect causing earlier spoilage of minced products from mackerel (*Scomber scombrus*), rainbow trout (*Oncorhynchus mykiss*) and cod (Richards and Hultin 2002). On whole fish blood residue has shown to cause an accelerated lipid oxidation in trout during frozen storage (Tretsven and Patten 1981). Similar to lipid oxidation, there is suggesting evidence that the hydrolysis of blood cells can promote proteolysis in the muscles accelerating the post mortem softening of the flesh (Ando and others 1999).

Although it is generally accepted that blood is negative to the shelf life and quality, there exist little scientific information on how blood affects the sensory attributes in fresh fish products. As the both the fat content and blood volume vary considerably amongst fish species ranging from 5.6 to 14 ml/kg (Ando and others 1999) or 1.5 to 7 % of body weight (Smith 1966), and the oxidative effect of blood depends not only on myoglobin and hemoglobin content, but also type (Richards and Hultin 2002; Grunwald and Richards 2006a, b), uncertainties raises whether blood residue will affect textural and sensory properties in bottom dwellers such as turbot.

The aim of this study is therefore to investigate the effect if blood residue and find relations between blood residue and its effect on the visual, textural and sensory properties

over a period of two weeks. In order to quantify and differentiate between levels of blood residue in the fillets, computer imaging system was used.

2. PART 3. Materials and methods

To identify what effect blood residue had on textural and sensory properties of farmed turbot, a total of 80 turbot with mean weight equal to $1.2 \text{ kg} \pm 0.17 \text{ SD}$ were on two occasions slaughtered at Silfurstjarnan, Iceland (n=60) (22 November 2005) and at A. Coelho e Castro, Portugal (n=40) (17 February 2006). The rearing temperature was 13.9 °C (Iceland) and 14.1 °C (Portugal).

2.1 Experiment 1: Sensory and visual appearance of blood measured by taste panel and computer imaging system (Iceland)

On day 1, to reveal what effect blood residue had on the sensory and visual quality of turbot three groups of fish were either:

1. Percussive killed and stored directly on ice unbled (Control) (n=10).
2. Percussive killed and electrical stimulated with pulsed direct current (pDC) for 3 min before placed on ice unbled (El-stim) (n=30).
3. Live exsanguinated by cutting of all both sets gill arches and placed into bins of ice slurry for 1 h bleed-out before placed in boxes of ice (Exsanguinated) (n=20).

Electrical stimulation was carried out to see whether the muscle contraction during stimulation would force the blood from the muscles. The electrical stimulation was carried out as according to Roth and others (2007) exposing the carcass with 20 V pulsed DC for 3 min.

As blood flows with gravity and settles on the lower fillet (Roth and others 2005), all fish were stored with the ventral side down in propylene boxes containing ice. Ten unbled fish stimulated with electricity (El-stim) and 10 bled fish (Exsanguinated) were sent by air cargo to Wageningen IMARES, The Netherlands for sensory evaluation. The sensory evaluation of bled and unbled fish was carried out using and trained taste panel performing Quantitative Descriptive Analyses (QDA) provided in more detail under the section on methods. The other 40 fish were transported to the Icelandic Fisheries Laboratory, Reykjavik and stored on ice until they were filleted on day 7. After filleting, each fillet from the dorsal back (upper and lower) were placed on a black base inside a photo box and photographed using a digital camera. Colour such as red, redness and whiteness from each fillet was quantified by computer imaging system described in more detail under the section of methods.

2.2 Experiment 2: Sensory and texture analysis of bled and unbled fish (Portugal)

On Day 1, in Portugal, a total of 40 fish were killed by a sharp blow to the head and 20 fish from each group was either exsanguinated (Bled) or not (Unbled). The exsanguination was carried out by cutting of both sets of gill arches and placed into ice-slurry for 30 min before the fish was stored into boxes with ice. The fish sent with air cargo to Norconserv A/S, Norway. On day 7, both the upper and lower fillet from the dorsal back was filleted off and used for measuring texture shear force (Kramer blade) and hardness (puncture test), separately. Further detail on texture measurements are described under the section on methods. For sensory analysis one fillet (lower ventral fillet) from each fish was wrapped into aluminium foil, stored in boxes with on ice and sent to Wageningen IMARES, The Netherlands for QDA analysis on day 14.

2.3 Computer imaging analysis

Computer imaging analysis was performed using a digital camera (Canon EOS 300d DIGITAL) and a standardized photo box. The photo box provided uniform and diffuse lighting on the scene. This was important to avoid reflexes on the moist and shiny fillet surfaces. Reflexes appear as bright white areas in an image and effectively remove any information from that part of the scene. The photo box also ensured that the lighting was identical from image to image ($T = 6500K$, $R_a \geq 90$). The output from the camera was RGB-colour-images. Each pixel (x,y) in an RGB-colour-image is a triplet corresponding to the intensity of the primary colours (R)ed, (G)reen and (B)lue at that point. The intensities were in the range $[0, 1, \dots, 255]$. The fully automatic image analysis of each image consisted of three steps: First, all light pixels where identified as fillet (1) and all others as background (0):

$$b(x, y) = \begin{cases} 1 & \Leftrightarrow (R(x, y) + G(x, y) + (B(x, y)))/3 > 50 \\ 0 & \text{otherwise} \end{cases}$$

The intensity of red was larger than the two other intensities for all pixels in the fillet regions. It was therefore decided to use this difference as a measure of how red the individual pixels were:

$$f(x, y) = (R(x, y) - (G(x, y) + B(x, y))/2) \cdot b(x, y)$$

Pixels with $f < 70$ were visually judged to have a whitish colour, pixels with $70 \leq f < 100$ as reddish and $f \geq 100$ as dark red. The following ratios where therefore calculated to describe the colour of the fillet region:

$$p(\text{white}) = 100 \sum_{k=1}^{69} n_k / n, \quad p(\text{reddish}) = 100 \sum_{k=70}^{99} n_k / n, \quad p(\text{red}) = 100 \sum_{k=100}^{255} n_k / n$$

where n_k is the number of pixels in f equal k and n is the number of pixels in $f > 0$.

2.4 Texture analysis

The texture analysis were carried out using a an TA-XT2® -pro Texture Analyzer from Stable Micro Systems, loaded with a 50 kg test cell. The puncture test was carried out on the upper dorsal fillet, while the Kramer blade shear force test was used on the lower dorsal fillet. For shear force measurements, one standards muscles sample (69×26 mm) from the loin was cut in the front and behind the midpoint of the fillet as according to Roth et al (2007). Each muscle sample was sliced at two locations with a 3×70 mm blade with 60° knifeedge with constant speed at 1 mm/s (Sigurgisladottir and others 1999, 2001), providing a total of 4 shear force samples on pr. fillet, where 1-4 represents from the anterior to the posterior (Roth and others 2007). For measuring texture hardness, a flat cylinder, 20 mm of diameter was used as the test probe. The penetration depth for the probe was 80 % of the fillet height holding a constant speed equal of 1 mm/s. The texture profile was sampled at 4 locations directly on the fillet along the loin. The location for sampling was standardized by taking two puncture tests in front of and two behind the midline of the fillet with 1 cm distance between each puncture test (Fig. 1) 4 shear force samples on pr. fillet, where 1-4 represents from the anterior to the posterior (Roth et al. 2007).

2.5 Sensory analysis

For sensory analyses of food products the QDA (also known as profile method) is common for characterization of the differences between products and to be able to provide sensory data for the interpretation of instrumental data. The method consists of procedures for describing and assessing the flavour of a product in a reproducible way. The separate attributes contributing to the formation of the overall impression given by the product are identified and their intensity assessed in order to build up a description of the flavour of the product. The QDA analyses were carried out according to IAO standard 6564 (1985, sensory analysis, methodology flavour profile methods). This panel consisted of 5 selected and trained persons for QDA of turbot. In previous sensory studies with turbot, 29 attributes were defined and described for raw as well as cooked turbot fillets. With the help of FIZZ for windows 2.10a biosystemes, the panelists scored on a line scale from 0-100. For the test artificial

daylight was used ($T > 5000\text{K}$). The samples were assessed in duplicate. Sample presentation order was randomized between panelists. Before sensory analyses the samples were filleted and cut in pieces of 2 by 4 cm. Raw samples were presented first on a plastic dish. For cooked evaluation the samples were placed in a glass dish with a lid and cooked in the microwave for 60 s (600 Watts). The samples were presented to the panel on a plastic dish immediately after cooking. Samples were coded with a three digit code.

2.6 Statistical analysis

The statistical analysis was performed on Statistica 7.1 and SAS 8.9. Factorial ANOVA was used to test the effects of the independent variables (bled vs. unbled) and (upper vs. lower fillet) on the colour percentages from the image analysis. For comparing the upper and lower fillet, originating from the same fish, a one-way paired t-test was applied. One way ANOVA was used to test the sensory attributes against the independent variables of bled and nonbled fish. Covariate analysis (ANCOVA) was used for testing independent and continuous variables as shear force and hardness against categorical variables such as treatment and fillet location, using fillet height as a covariate. For post hoc analysis Tukey test were used. The level of significance was set $P < 0.05$.

3. PART 3. Results

3.1 Computer imaging system

As shown in Figure 7 and Table 5, the fillet whiteness and redness was evaluated by computer imaging of photographs was dependent on the blood residue in the fillet depending whether the fish had been exsanguinated ($P < 0.0005$, factorial ANOVA) and the orientation of the fillet ($P < 0.005$, factorial ANOVA). Computer imaging system was very accurate in detecting differences in blood residues and could distinguished between the upper and lower fillets even amongst exsanguinated fish ($P < 0.0005$, one way paired t-test). Quantifying redness as the proportion area with $\text{Cr} > 100$ reveals significant difference between bled and non bled fish ($P < 0.05$, factorial ANOVA), but not fillet orientation ($P > 0.05$, factorial ANOVA).

Table 5. Whiteness and Red (%) provided as mean \pm SE in turbot fillets originating from bled (Exsanguinated) and unbled (Control and El-stim) fish. The orientation of the fish was accounted from slaughter where the fish was stored with the ventral side down. In each column, different capital letters X,Y represent a significant difference of $P < 0.05$ (ANOVA). In each row different lowercase letters represents significant differences at $P < 0.05$ (paired t-test).

	Colour mean (SE)				n
	<i>p</i> (white)		<i>p</i> (red)		
	Lower fillet	Upper fillet	Lower fillet	Upper fillet	
Control (Unbled)	73.8 (4.11) ^{aX}	91.7 (2.30) ^{bX}	0.66 (0.18) ^{aX}	0.09 (0.06) ^{bX}	10
El-stim (Unbled)	81.0 (3.11) ^{aX}	91.9 (1.68) ^{bX}	0.56 (0.21) ^{aX}	0.18 (0.12) ^{bX}	20
Exsanguinated (Bled)	96.3 (0.38) ^{aY}	98.6 (0.24) ^{aY}	0.02 (0.01) ^{aX}	0.001 (0.001) ^{bX}	10

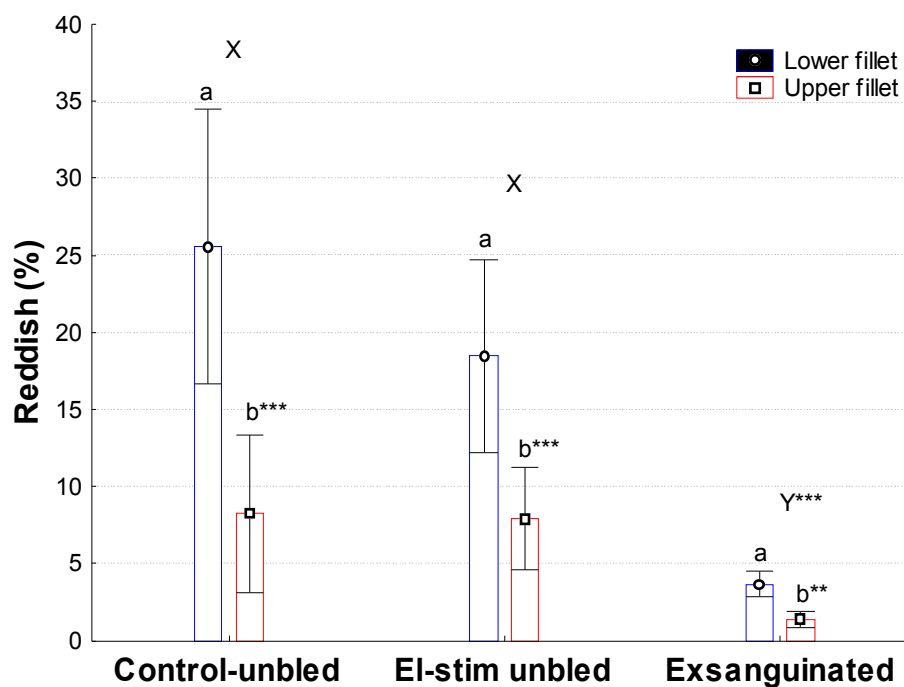


Fig. 7. Reddishness (%) provided as mean \pm SE in turbot fillets originating from bled (Exsanguinated) and unbled (Control and El-stim) fish. The orientation of the fish was accounted from slaughter where the fish was stored with the ventral side down. Different capital letters X,Y,Z represent a significant difference between groups (ANOVA) and different lowercase letters represent significant difference between upper and lower fillet within a group (paired t-test) of * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

3.2 Texture analysis

Results from Kramer shear force analysis (Table 6) show no significant differences between bled and non bled fish originating from Portugal ($P > 0.129$, factorial ANOVA), but the shear force was dependent on the location on the fillet, increasing while sampling backwards towards the caudal part of the fillet ($P < 0.05$, factorial ANOVA). As with shear force, the puncture test revealed no significant difference in hardness between bled and unbled fish ($P > 0.15$, ANCOVA), but the hardness did vary accordingly to where on the fillet the

puncture was taken ($P < 0.005$, ANCOVA) and fillet thickness ($P < 0.05$, ANCOVA).


Table 6. Maximum shear force (N) recorded in while slicing standards muscled samples cut from turbot fillet providing in total 4 samples pr. fillet. There were no significant difference in shear force between bled and unbled fillets, while different letters represent significant difference between sampling location (1-4) of $P < 0.05$.

	Fillet location	Shear Force (N)		N
		Mean	SE	
Unbled	1	71.0 ^{a,b}	3.75	20
	2	69.7 ^b	2.95	20
	3	84.2 ^{a,b}	4.74	20
	4	86.5 ^a	3.67	20
Bled	1	67.3 ^a	3.10	20
	2	70.6 ^a	4.90	20
	3	74.9 ^a	3.67	20
	4	82.0 ^a	3.67	20

3.3 Sensory analysis

After 7 of storage the taste panel was unable to distinguished differences amongst bled and unbled fish texture or in taste. However the taste panel could detect a difference in the raw appearance, where muscle samples from exsanguinated fish appeared whiter and unbled fish had a more greyish appearance. Increasing the storage time to 14 days on ice, whereas 7 of these days the fish were stored as single fillets, did show no significant differences between bled and unbled fish (Table 7).

Table 7. Quantitative Descriptive Analyses of raw and cooked samples from bled and unbled turbot. Used was a trained taste panel (n=6) where the fish were stored for 14 days (7 d were stored as fillets). Number of fillets from each group was 10 and samples pooled and presented to the panel in replicates of two (N=12). There were no significant differences between bled and unbled fish in any of the attributes neither in raw or cooked samples.

	Attribute	Mean Score	
		Bled	Unbled
Raw samples			
Appearance	Cream	32.42	39.08
	Glass	24.08	29.33
	Gray	26.42	24.17
Odor	Potato	25.75	29.50
	Hay	9.33	16.50
	Marine	19.67	15.58
	Mustard	7.17	9.08
	Sour	14.08	21.58
Cooked samples			
Appearance	Creamy	31.83	34.00
	Gray	17.08	19.42
	Grabby	12.42	24.83
Odor	Milk	46.92	47.83
	Hay	21.67	22.58
	Mustard	15.75	14.33
	Cardboard	15.50	16.33
	Sour	12.00	17.33
	Fishy	33.58	30.83
Texture	Firm	50.67	52.17
	Tender	59.00	58.42
	Fibrous	44.33	51.08
	Granular	18.83	24.83
	Sticky	33.25	34.00
	Dry	25.25	33.17
	Taste	Creamy	31.08
Potato		39.67	39.33
Chicken		23.50	28.58
Stock solution		18.42	18.25
Water			
		61.92	61.75
Sour		20.08	24.25

4. PART 3. Discussion

As expected, the exsanguinated fish contained far less blood than unbled fish and the blood was visual for the human eye. Using Computer imaging system to quantify the amount of blood seen in the fillet did show high accuracy in distinguishing differences between treatments and orientation of the fillet. Previous quality studies on salmonids show that the blood content can be quantified either visually by counting the number of bloodspots seen after salting and smoking (Robb and others 2003; Roth and others 2005) or measuring the haemoglobin (Hb) content within the fillet (Olsen and others 2006). Furthermore, as the fish is stored, the blood will flow with gravity, accumulating in the lower fillet (Roth and others 2005). In these studies differences between bled and unbled fish could be statistically verified from $P < 0.05$ to $P < 0.005$, depending number of fish and methods used. Computer imaging system did however detect differences between bled and unbled fish at a level of $P < 0.00001$, and even $P < 0.005$ between the upper and lower fillet from exsanguinated fish. This truly reveals the potential of using computer vision as a tool for detecting and quantifying blood in whitefish. Counting bloodspots involves quantifying the blood residue in veins, but not capillary blood and vice versa when measuring haemoglobin in small white muscle samples. Computer visions do however quantify the total amount of blood across the whole fillet and would therefore provide a more reliable picture on the amount of blood present. Also, using colour for quantification it is possible to predict the oxidative state of blood as the colour changes from red to dark brown or black, depending on oxidative state.

Still when exsanguinating live fish, poor bleed-out and high amount of blood in the fillet do occur. Besides bleeding method (Botta and others 1986; Robb and others 2003) and timing of exsanguination (Valdimarsson and others 1987; Roth and others 2005), little is known on what factors that might affect bleed-out. Olsen and others (2006) reported that stressed fish contained higher *post mortem* haemoglobin values in white muscle tissue, and this within reason as stress and high muscle activity, *pre mortem*, will a redirection of intestinal blood to the muscles (Thorarensen and others 1993; Farrell and others 2001). However little is known how muscle activity and the onset of *rigor mortis* affect the blood distribution in the fillet. As shown in Figure 1 electrically stimulated fish had neither more nor less blood in the fillet despite substantial *post mortem* muscle activity and a much earlier onset of *rigor mortis* (Roth et al. 2007). Theoretically, with a cardiac arrest and halted circulatory system, the internal tensions during muscle contractions should force the blood from the muscles and into the large aorta and veins along the spinal column and into the gut,

but this seems not to be the case.

Although blood is generally assumed to be detrimental to the shelf life and quality for fish, little is known how blood affects the shelf life in a fresh product. As shown in Table 3 the blood residue in turbot fillets had apparently no effect on the freshness over a 14 days storage period. This is clearly different from what was expected were fillets from unbled fish should accordingly become rancid due to lipids oxidation, catalyzed by hemolysis and formation of superoxide, hydrogen peroxide and hydroxyl radicals (Misra 1972; Puppo and Halliwell 1988a, b; Baron and Andersen 2002; Grunwald and Richards 2006a, b). Previously reports show that elevated levels of hemo- and myoglobin does cause an earlier spoilage of minced mackerel, rainbow trout (*Oncorhynchus mykiss*) and cod (Richards and Hultin 2002). In whole fish, it has been shown that pro oxidative activity is associated with the haemoglobin content and the following rancidity has also been demonstrated during frozen storage of both trout (Tretsven and Patten 1981) and mackerel (Richards and others 1998). Although several recent studies has been adding Hb, Mb or hemin directly to homogenized, washed and sterile cod muscle, identifying the relationship between the presence of hemin and lipid oxidation, the practical relevance and specie dependency is not fully exploited.

It is commonly known that the main components for spoilage of very fatty species, such as herring and mackerel, is lipid oxidation, while bacterial contamination is the main source for rejecting lean fish, such as Atlantic cod. Turbot can be considered as partly lean specie, has an exceptional long shelf life laying in the region of 20-30 days (Campos and others 2006; Rodriguez and others 2006). Our storage period of 14 days may seem premature, but notably half of this storage period the fish were stored as fillets and the fat, muscles and blood were exposed to air and bacterial contamination. Apparently the blood residue had no effect whatsoever when presented for an expert taste panel. Reason for this is unclear, but there is suggesting evidence that the reason may lay in characteristics of the blood. Being dermal fish, the volume in flatfish is half compared to pelagic species, and when exsanguinating the fish, the blood cell count in the muscle tissue is 400 to 600 % lower than pelagic species (Ando and others 1999). Furthermore, the pro oxidative effect of Hb varies considerably between different fish species, where the Hb form in winter flounder (*Pleuronectes americanus*) is considerably less catalytic than Hb found in mackerel and pollack (*Pollachius virens*) (Undeland and others 2004). Considering all factors associated to turbot as fat content, blood volume, cell count and Hb type could explain why there were no textural or sensory differences between bled and non bled fish. This could partly explain why flat fish in general do have an extensive shelf life compared to other fish species.

Concluding remarks and increased value of sea products

We conclude that over a period of 14 days, the blood residue in turbot fillets only affects the visual appearance, but not the eating quality. Furthermore we conclude that computer imaging system is an interesting tool for rapidly quantifying the amount of blood in whitefish, and its potential should be further explored.

Colour of the fillets can be tuned by (post) slaughter conditions. Non-bleeding conditions will result in a more crème colour of the fillet what is more like wild turbot. These findings are not patentable, but they contribute to increased farming knowledge of turbot and are of clear practical interest for turbot farmers. Firstly, it is important to have a clear view of how post slaughter methods affect the final product. The knowledge obtained in this study will be beneficial for the company involved (Silfurstjarnan) as for the whole Icelandic farming industry, as increased knowledge on how post slaughter conditions affect the final product will help the farming industry to increase the value of their farming product. Overall, the methodological development employed in this project will increase the value of the product (here, slaughter size turbot) thereby leading to an increased value of sea products in line with AVS main objective.

It is suggested to validate the findings presented here for other farmed species, as producers must know the effect of blood residue and its effect on the visual, textural and sensory properties of their farmed product. The use of computer imaging system is a promising tool and should be considered for use in future studies of the same kind

5. Part 3. Acknowledgements

These experiments was a part of the EC-CRAFT project no. 508070, TURPRO and AVS project R032-05 We would like to thank all the involved project partners A.Coelho e Castro, Portugal; Ecomares, Germany; Llyn Aquaculture Ltd, U.K; Silfurstjarnan, Iceland; and Zeeland Vis BV, Netherlands. Also thanks to Soffía Vala Tryggvadóttir at the Icelandic Fisheries Laboratory, Iceland and Sigurd Øines ved Norconserv A/S, Norway and Mette Remen at University of Bergen for technical support during the experiment.

6. PART 3. References

- Ando M, Nishiyabu A, Tsukamasa Y, Makinodan Y. 1999. Post-Mortem Softening of Fish Muscle During Chilled Storage As Affected by Bleeding. *J Food Sci* 64(3):423-428.
- Baron CP, Andersen HJ. 2002. Myoglobin-Induced Lipid Oxidation. A Review. *Journal of Agricultural and Food Chemistry* 50(14):3887-3897.
- Botta JR, Squires BE, Johnson J. 1986. Effect of Bleeding Gutting Procedures on the Sensory Quality of Fresh Raw Atlantic Cod (*Gadus-Morhua*). *Can Inst Food Sci Technol* 19(4):186-190.
- Campos CA, Losada V, Rodriguez O, Aubourg SP, Barros-Velazquez J. 2006. Evaluation of an Ozone-Slurry Ice Combined Refrigeration System for the Storage of Farmed Turbot (*Psetta Maxima*). *Food Chem* 97(2): 223-230.
- Farrell AP, Thorarensen H, Axelsson M, Crocker CE, Gamperl AK, Cech JJ. 2001. Gut Blood Flow in Fish During Exercise and Severe Hypercapnia. *Comp Biochem Physiol* 128(3):551-563.
- Grunwald EW, Richards MP. 2006a. Mechanisms of Heme Protein-Mediated Lipid Oxidation Using Hemoglobin and Myoglobin Variants in Raw and Heated Washed Muscle. *Journal of Agricultural and Food Chemistry* 54(21):8271-8280.
- Grunwald EW, Richards MP. 2006b. Studies With Myoglobin Variants Indicate That Released Hemin Is the Primary Promoter of Lipid Oxidation in Washed Fish Muscle. *Journal of Agricultural and Food Chemistry* 54 (12):4452-4460.
- Misra HP. 1972. Generation of Superoxide Radical During Autoxidation of Hemoglobin. *Journal of Biological Chemistry* 247(21):6960-&.
- Olsen SH, Sorensen NK, Stonno SK, Elvevoll EO. 2006. Effect of Slaughter Methods on Blood Spotting and Residual Blood in Fillets of Atlantic Salmon (*Salmo Salar*). *Aquaculture* 258(1-4):462-469.
- Puppo A, Halliwell B. 1988a. Formation of Hydroxyl Radicals From Hydrogen-Peroxide in the Presence of Iron - Is Hemoglobin A Biological Fenton Reagent. *Biochemical Journal* 249(1):185-190.
- Puppo A, Halliwell B. 1988b. Formation of Hydroxyl Radicals in Biological-Systems - Does Myoglobin Stimulate Hydroxyl Radical Formation From Hydrogen-Peroxide. *Free Radical Research Communications* 4 (6):415-422.
- Richards MP, Hultin HO. 2002. Contributions of Blood and Blood Components to Lipid Oxidation in Fish Muscle. *Journal of Agricultural and Food Chemistry* 50(3):555-564.
- Richards MP, Kelleher SD, Hultin HO. 1998. Effect of Washing With or Without Antioxidants on Quality Retention of Mackerel Fillets During Refrigerated and Frozen Storage. *Journal of Agricultural and Food Chemistry* 46(10):4363-4371.
- Robb DHF, Phillips AJ, Kestin SC. 2003. Evaluation of Methods for Determining the Prevalence of Blood Spots in Smoked Atlantic Salmon and the Effect of Exsanguination Method on Prevalence of Blood Spots. *Aquaculture* 217(1-4):125-138.
- Rodriguez O, Barros-Velazquez J, Pineiro C, Gallardo JM, Aubourg SP. 2006. Effects of Storage in Slurry Ice on the Microbial, Chemical and Sensory Quality and on the Shelf Life of Farmed Turbot (*Psetta Maxima*). *Food Chem* 95(2):270-278.
- Roth B, Torrissen OJ, Slinde E. 2005. The Effect of Slaughtering Procedures on Blood Spotting in Rainbow

- Trout (*Oncorhynchus Mykiss*) and Atlantic Salmon (*Salmo salar*). *Aquaculture* 250(3-4):796-803.
- Sigurgisladottir S, Hafsteinsson H, Jonsson A, Lie O, Nortvedt R, Thomassen M, Torrissen O. 1999. Textural Properties of Raw Salmon Fillets As Related to Sampling Method. *J Food Sci* 64(1):99-104.
- Sigurgisladottir S, Sigurdardottir MS, Ingvarsdottir H, Torrissen OJ, Hafsteinsson H. 2001. Microstructure and Texture of Fresh and Smoked Atlantic Salmon, *Salmo salar* L., Fillets From Fish Reared and Slaughtered Under Different Conditions. *Aquacult Res* 32(1):1-10.
- Smith LS. 1966. Blood Volumes of Three Salmonids. *Journal of Fisheries Research of Board of Canada* 23 (1775-1790).
- Thorarensen H, Gallagher PE, Kiessling AK, Farrell AP. 1993. Intestinal Blood-Flow in Swimming Chinook Salmon *Oncorhynchus tshawytscha* and the Effects of Hematocrit on Blood-Flow Distribution. *J Exp Biol* 179(115-129).
- Tretsven WI, Patten BG. 1981. Effect of Arterial Incisions on the Amount of Bleeding and Flesh Quality of Rainbow-Trout. *Marine Fisheries Review* 43(4):16-18.
- Undeland I, Kristinsson HG, Hultin HO. 2004. Hemoglobin-Mediated Oxidation of Washed Minced Cod Muscle Phospholipids: Effect of PH and Hemoglobin Source. *Journal of Agricultural and Food Chemistry* 52(14): 4444-4451.
- Valdimarsson G, Matthiasson A, Stefansson G. 1987. Bleeding and Gutting of Cod No 5325, 13pp. *Can Trans Fish and Aquatic Sci.*