Holding of Arctic char in a RAS transport system

Guðmundur Stefánsson

Aðalheiður Ólafsdóttir

Skýrsla Matís 22-19

Desember 2019

ISSN 1670-7192

DOI 10.5281/zenodo.3581202

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	**Holding of Arctic char in a RAS transport system**		
Höfundar / Authors	Guðmundur Stefánsson and Aðalheiður Ólafsdóttir		
Skýrsla / Report no.	22-19	*Útgáfudagur / Date:*	December 2019
Verknr. / Project no.	62523		
Styrktaraðilar /Funding:	EIT Food		
Ágrip á íslensku:	Í september 2019 voru tvær tilraunir framkvæmdar í að halda bleikju (*Salvelinus alpinus*) lifandi í RAS kerfi sem þróað var af Technion, Ísrael. Kerfið, sem hringdælir vatni, stýrir sýrustigi (pH) og fjarlægir skaðleg ammóníakssambönd, var sett upp í kæligámi til að halda hitastiginu við 4°C. Verkefnið var samstarf Technion og Matís og styrkt af EIT Food. Niðurstöður sýna að hægt var að halda 80 kg/m^3 af bleikju í kerfinu í alla vega 8 daga við 4°C, án teljandi áfalla (um 4% dauðatíðni). Engin munur fannst á skynrænum gæðum (bragði, lykt, útliti og áferð) á fiski úr RAS kerfinu í samanburði við fisk sem ekki var settur í kerfið. Meira los sást þó í holdi fisks úr RAS kerfinu, meiri suðunýting og lítillega ljósari litur. Hins vegar, þegar magn bleikju í kerfinu var aukið í 135-145 kg/m^3 drapst megnið af fiskinum. Við slátrun hafði sá fiskur er lifði af, minni skynræn gæði en fiskur áður en hann fór í kerfið, með tapi á einkennandi bragði og lykt og þéttari, þurrari og seigari áferð. Fiskurinn úr kerfinu hafði auk þess meira los, meiri suðunýtingu og bitar aflögðuðust við suðu.		
Lykilorð á íslensku:	*Bleikja, RAS kerfi, flutningur á lifandi fiski, gæðamat*		

Summary in English:	In September 2019 two live holding trials with Arctic char (*Salvelinus alpinus*) were carried out at Matís where the fish was kept for up to eight days in a RAS holding and transport system developed by Technion, Israel Institute of Technology. The RAS system, which recirculated the water, controled the pH and removed accumulated ammonia, was set up in a 40 feet reefer tank to control the temperature at 4°C. The project was funded by EIT food and the participants were Technion and Matís. The results show that Arctic char could be held at a density of 80 kg/m^3 at 4°C for 8 days in the RAS system, without adverse effects on mortality. Moreover, no differences were found in the sensory quality (flavour, odour, appearance and texture) of the stored fish compared with fish before it was placed in the RAS system. The stored fish had however more gaping, higher cooking yield and marginally lighter colour than fish before placing in the system. However, a bio-load of 135-145 kg/m^3 Arctic char in the RAS storage and holding system led to a high mortality. Moreover, on slaughter the surviving fish had adverse sensory quality as indicated by loss of characteristic flavour and odour as well as firmer, drier and tougher texture. The fish had a high incidence of gaping, a high cooking yield and showed evidence of deformation on cooking.
English keywords:	*Arctic char, RAS system, live transport and storages, quality evaluation*

Table of Contents

1. Introduction 5
2. Materials and methods 5
   2.1 Live fish 5
   2.2 Quality evaluation methods 7
   2.3 Other analysis 8
   2.4 Data analysis 8
3. Results and discussion 10
4. Conclusion 14
5. Acknowledgements 14
6. References 15
7. Appendix 16

1. Introduction

In September 2019, two live Arctic char holding trials were carried out at Matís where the fish was kept for up to eight days. The idea behind the trials was to simulate holding and transport of live fish from Iceland to potential live fish markets (e.g. restaurants) in Europe. The transport of live seafood is challenging, expensive and difficult, due to factors such as oxygen supply, increase of carbon dioxide in the water, accumulation of toxic ammonia and changes in pH.

In our trials we used the RAS holding and transport system developed by Technion and BioFishency (www.biofishency.com) which not only recirculates the water, but additionally controls the pH and removes accumulated ammonia. The system was set up in a 40 feet reefer tank to control the temperature and was connected to two 1 m^3 fish tanks. The aim of the trials was to test two densities of Arctic char, approximately 80 kg/m^3 and 120 kg/m^3.

The project was funded by EIT food with Technion leading and Matís participating.

2. Materials and methods

2.1 Live fish.

Before starting the trial, a permission to carry out live holding trials with Arctic char (*Salvelinus alpinus)* was obtained from MAST (see Appendix). The score sheet for scoring endpoints in Arctic char can also be found in the Appendix.

The Arctic char was provided by the Samherji farms in Grindavík (Staður). The fish was grown in about 23-24 pro mille salt at a temperature of 7-8°C. The fish used for the holding trials was intended for slaughter and markets in USA. For the first trial, the Arctic char had been starved for 12-14 days and for the second trial it was starved for 10 days. The fish used for the trials was specifically picked out from the slaughtering line as “small“ fish. The weight range of the fish for slaughter was expected to be between 1.300 to 1.600 g and the average weight of fish for our trials were 1.250 g (*Trial 1*) and 1.240 g (*Trial 2*). However, considerable individual weight range was found for the fish or from approximately 590 g to 2.000 g. The fish was transported from Samherji, Grindavík in large tubs aerated with oxygen, a journey that took about one to two hours (Figure 1).

Figure 1. Tubs and the system used to transport Arctic char from Grindavík to Matís

For *Trial one* approximately 130 kg of fish were delivered to MARS (Keldnaholt, Reykjavík) on the 16th of September 2019; thereof about 120 kg used for the trial (approx. 59 kg in Tank 1 and 61.8 kg in Tank 2). For trial two about 255 kg of Arctic char were delivered to MARS on the 26th of September and thereof approximately 230 kg were used for the trials (about 119 kg for Tank 1 and 112 kg for Tank 2). It should be noted that the two holding tanks were connected so they served as a duplicate. To transfer the fish into the 2 x 1 m^3 tanks, first the sea water from the transport tubs was pumped into the tanks. The fish was then transferred in batches, using a large fishing net into 10 litre boxes with the seawater, weighed and then transferred into the tanks.

Figure 2. Arctic char transferred to the RAS tanks used for the trials

Fish that died due to stress related issues from transport and handling were removed from the tanks after one day in the RAS system, which marked the end of the acclimation period. The temperature in the reefer was slowly reduced to 4°C (about 1 degree/day). In *Trial 1* the density or bio-load of fish was about 80 kg/m^3 in the tanks. In *Trial 2*, the density was about 135-145 kg/m^3 in the tanks or approximately 15-20% higher than the original plan (120 kg/m^3). In *Trial 1* the Arctic char was kept in the system for 8 days. In the second trial the plan was to hold the fish for 10 days, but the trial was terminated early (on day 7) due to stress related symptoms and the fish was slaughtered.

At slaughtering, the fish were removed from the system, struck on the head with a stunner and bled in circulating iced water. After bleeding, the fish were iced in boxes and brought to Matís where the

fish were eviscerated and cleaned and kept in ice and in a chiller at 0-2°C until the fish were filleted and used for testing. Ten fish were slaughtered at the beginning of the trials to evaluate the initial quality (sensory evaluation, colour analysis and proximate analysis). Fish slaughtered at the beginning of the second experiment (on the 26th of September; group *Initial fish*) were used for comparison with the fish that had been kept in the system for eight days (*Trial 1*). In *Trial 2*, most of the fish had died after seven days in the RAS system. However, the rest of the fish was slaughtered, and ten fish used for the evaluation and compared with the group, *Initial fish*.

2.2 Quality evaluation methods

The following methods were used for the evaluation: GDA (Generic Descriptive Analysis), cooking yield, analysis of fillet gaping, colour analysis and proximate analysis (moisture, protein, fat, ash and salt content).

Sensory evaluation: The sensory method, Generic Descriptive Analysis (GDA, Lawless and Heymann, 2010) was used to analyse cooked samples of Arctic char. Seven panellists participated in the sensory evaluation. All panellists had been trained according to international standards (ISO 8586, 2014); including detection and recognition of tastes and odours, use of scales and in the development and use of descriptors. The members of the panel were experienced in using the GDA method. The intensity of each attribute for a given sample was evaluated using a 15 cm unstructured scale which in analysis was transformed to numbers from 0 to 100. All attributes were defined and described by the sensory panel during earlier projects. The sensory attributes were 20 and are described in Table 1. Two training sessions were carried out prior to the analysis in order to harmonise the panellists´ use of the attribute scale. The Arctic char were stored whole on ice at 0-2°C but filleted and skinned just before each evaluation. For GDA, nine fillets from different individuals were used for each sample group per sampling day. Portions weighing about 40-50 g were cut transversally from the fillets and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100°C with air circulation and steam, and then served warm to the panel. Each panellist evaluated triplicates of each sample group in a random order (three samples per session). A computerised system (FIZZ, Version 2.51C, 1994-2018, Biosystémes) was used for data recording.

Moisture content: Water content was determined by the difference in weight of homogenized muscle samples before and after drying for 4 h at 102 to 104 °C (ISO 1999). Results were calculated as g water per 100 g muscle.

Salt content: was determined by the method of Volhard according to the AOAC Official Methods of Analysis (1995).

Protein content of the fish muscle was estimated by the Kjeldahl method (ISO 1997) with the aid of a Digestion System 40 (Tecator AB, Hoganas, Sweden) and calculated using total nitrogen (N) x 6.25.

Total lipids of the muscle samples were extracted according to the method of Bligh and Dyer (1959). The lipid content was determined gravimetrically. The results were expressed as g lipid per 100 g of the muscle.

Colour evaluation: Ten fillets from different individuals were used for analysis of colour and gaping. Fillet colour CIE L*a*b* was measured by image analysis DigiEyeTM (VeriVide Ltd, Leicester, UK). Each fillet was placed into an illumination cabinet which ensures a uniform lighting, standard daylight (6400 K) and photographed with a Nikon camera D80 with a Nikkor lens. The colour of each sample was measured at a defined area in the posterior muscle using DigiPix colour measurement software.

Gaping was evaluated on a seven-point scale from none to very high gaping. Industrial scale photos were used as a reference (Erikson, 2009). The gaping scale and reference photos are shown in the Appendix. Five panellists participated in the gaping evaluation.

Cooking yield was evaluated on one identical sample (ca. 50-100 g) which was cut transversally from each of ten fillets, the same as those used for sensory evaluation (GDA). Each sample was weighted before and after cooking for eight minutes in steam and cooling at room temperature for 30 minutes. The yield after cooking was calculated as the ratio of the sample weight after cooking to the sample weight before cooking as presented by:

$$Cooking\ yield\ of\ fillets(\%) = \frac{g\ cooked\ sample}{g\ raw\ sample} \times 100$$

Total plate counts (TPC) was determined in the recirculating water by a convential "pour-plate" method on Plate Count Agar. Incubation temperature was at 22°C (72 hours) for psychrotrophic bacteria (NMKL 184, 2006).

2.3 Other analysis

Analysis on pH, alkalinity, total ammonia nitrogen (TAN), oxygen saturation and temperature in the recirculating seawater were carried out and the results are presented in a separate report prepared by Technion.

2.4 Data analysis

For sensory data, that is GDA and gaping evaluation, *Initial fish* was compared separately with fish from *Trial 1* on one hand and *Trial 2* on the other hand. This was done because of difference in panel composition between the two experiments. A better comparison is achieved when using data from panellists who evaluate all samples in an experiment. Both comparisons for GDA are based on six

panellists. For gaping, comparison between *Initial fish* and *Trial 1* is based on four panellists and comparison between *Initial fish* and *Trial 2* is based on three panellists. The sensory evaluation program Panelcheck V1.3.2 (Nofima, Tromsø, Norway) was used to assess panel performance. The program NCSS 2000 (NCSS, Utah, USA) was used for statistical analysis of the results. For sensory data, analysis of variance (ANOVA, General linear model method) was used to compare groups and correct for difference in panellists´ use of the scale. One Way Anova was used to compare groups for cooking yield and colour data. Duncan´s test was used to perform multiple comparisons between groups. The significance level was set at 5%.

Table 1. Sensory attributes for Arctic char, scale anchors, and definitions.

Sensory attribute	Short name	Scale	Definition
ODOUR			
sweet characteristic	O-sweet	none || much	Sweet characteristic odour of boiled trout
metallic	O-metallic	none || much	Metallic odour
fresh fishoil	O-fishoil	none || much	Odour of fresh unspoiled fish oil
acidic	O-acidic	none || much	Citric acid, not spoilage sour
earthy	O-earthy	none || much	Earthy odour
rancid	O-rancid	none || much	Rancid odour, spoilage characteristic
APPEARANCE			
white precipitation	A-precipit.	none || much	White precipitation on the sample surface and/or between flakes in sample
heterogenous colour	A-heterog.	none || much	On the sample surface, how heterogenous is the colour
colour	A-colour	light || dark	Inside sample; white / orange colour
fat droplets	A-fat dropl.	none || much	Amount of fat in liquid surface.
FLAVOUR			
sweet characteristic	F-sweet	none || much	Sweet characteristic flavour of boiled trout
metallic	F-metallic	none || much	Metallic flavour
fresh fishoil	F-fishoil	none || much	Flavour of fresh unspoiled fish oil
acidic	F-acidic	none || much	Citric acid flavour, not spoilage sour
earthy	F-earthy	none || much	Earthy flavour
rancid	F-rancid	none || much	Rancid flavour spoilage characteristic
TEXTURE			
soft	T-soft	firm || soft	Evaluated in first bite
juicy	T-juicy	dry || juicy	Juicy - releases liquid when chewing, dry- draws liquid from mouth
tender	T-tender	tough || tender	Evaluated while chewing
mushy	T-mushy	little || much	Mushy texture, puree,
sticky	T-sticky.	none || much	Sticky texture, force needed to pull teeth apart after biting

3. Results and discussion

Table 2 shows the number of fish, the weight and the mortality of the Arctic char in the two trials. The results show that only 2%-6% (4% on average in the two tanks) of the Arctic char died during an 8-day storage at 80 kg/m^3 density. The fish was in good condition on slaughter.

Table 2. Number, weight and mortality of Arctic char held for either 7 or 8 days at 4°C in the RAS storage and holding system at two different densities.

	Trial 1 (8 days)		Trial 2 (7 days)	
	Tank 1	Tank 2	Tank 1	Tank 2
Fish in system (number)	48	47	91	83
Weight (kg)	61,3	60	110,8	103
Density (kg/m^3)	83	81	145	135
Mortality	6%	2%	87%	96%
Average weight (kg)	1,3	1,3	1,2	1,2

In the second trial we increased the density of Arctic char to about 135-145 kg/m^3 (*Trial 2*) and planned to hold the fish for 10 days. On day one, dead fish was removed from the system; seven from tank 1 and ten from tank 2 and the acclimation period was completed. The temperature of the recirculating water was still quite high or about 8°C, and the fish appeared agitated, so we reduced the temperature rapidly and increased the air flow to reduce CO_2 build up in the system. By increasing the air flow and decreasing the temperature the fish in the tanks appeared calm. However, as one fish was found dead on the floor of the reefer on day six, we decided to stop the trial early. On day seven, the trial was terminated but at this stage most of the fish had died and sunk to the bottom of the tanks. It is likely that we put too many fish in the RAS system in *Trial 2* and that the fish died of stress related symptoms rather than by a failure in the operation of the RAS system. It has been reported that Arctic char can tolerate grow-out densities of up to 130 kg/m^3 (Summerfelt at al., 2004). We surpassed that level by 5-10% and it may have been too much for the Arctic char in the trial.

The total Aerobic Count was higher in the recirculating water at the start of *Trial 2* compared with *Trial 1* and remained higher at all sampling points (Figure 3).

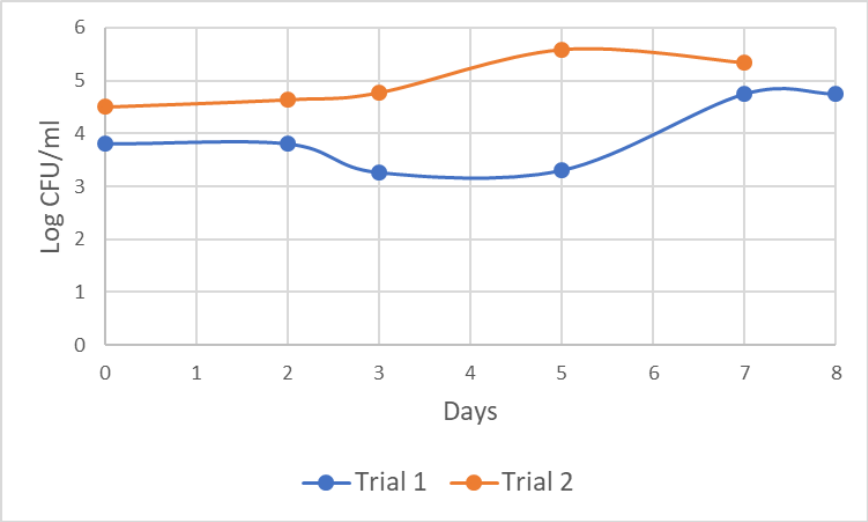

Figure 3. Total Aerobic Count in the RAS system for the Arctic char trials

Table 4 shows the proximate composition of the fish used in the trials both before placing it in the holding system and at end of the storage period. Considerable variation is seen in the content of fat and moisture. As only a few samples were taken for proximate analysis, no statistical analysis could be performed and the differences observed may simply be due to individual variation between fish, rather than related to the trial set-up. Still, the low moisture content observed in *Trial 2* may indicate stress-related changes.

Table 3. Proximate composition of the Arctic char used for the holding and storage trials.

	Protein	Fat	Water	Ash	Salt
Fish at start (D0)	17,8%	24,0%	57,1%	1,1%	0,1%
Fish at end (D8)	18,8%	19,8%	60,7%	1,1%	0,1%

Fish at start (D0)	19,3%	15,6%	63,8%	1,2%	0,1%
Fish at end (D7)	20,8%	22,1%	55,5%	1,3%	0,1%

The results from GDA are shown in Table 4. *Initial fish* is compared with fish at end of *Trial 1* on one hand and slaughtered fish from *Trial 2* on the other hand. For each comparison, only results from panellists who evaluated all samples from both groups are used. In both cases, the comparison is based on results from six panellists.

No differences were seen between the groups *Initial fish* and fish at end of *Trial 1*. However, a big difference was seen between groups *Initial fish* and *Trial 2* in odour, flavour, appearance and texture. The *Initial fish* group had a sweeter odour, more metallic odour and flavour and more fresh fish oil flavour than slaughtered fish at end of *Trial 2*. More white precipitation and less fat in cooking liquid was seen in fish from *Trial 2*. *Initial* fish group was much softer, more tender and juicier than

slaughtered Arctic char at end of *Trial 2*. A few comments from panellists were made on samples from *Trial 2* as being very dry and having a crumbly texture.

Initial fish group had less gaping than fish both from *Trial 1* and *Trial 2*. An average gaping score for group *Initial fish* was 0,4. Average values for groups *Trial 1* and *Trial 2* were 1,3 and 1,6 respectively (p-value < 0,000 for comparison between *Initial fish* and *Trial 1*, p-value = 0,011 for comparison between *Initial fish* and *Trial 2*).

Table 4. Mean values for GDA sensory attributes for cooked Arctic char. Comparisons between *Initial fish* (before RAS) with end of *Trial 1* (8 days) and *Trial 2* (7 days)

Sensory attribute	*Initial*	*Trial 1*	p-value	*Initial*	*Trial 2*		p-value
ODOUR							
sweet characteristic	53	52	0,818	50	35	**	0,003
metallic	25	27	0,231	21	11	*	0,046
fresh fish oil	15	17	0,506	12	10		0,120
acidic	4	5	0,129	3	5	ms	0,083
earthy	6	8	0,471	5	11	ms	0,066
rancid	1	1	0,102	0	2		0,101
APPEARANCE							
white precipitation	15	17	0,425	17	27	*	0,030
heterogenous colour	20	20	0,904	24	28		0,423
colour	49	53	0,532	50	51		0,829
fat droplets	60	53	0,381	62	40	*	0,011
FLAVOUR							
sweet characteristic	53	51	0,494	53	36		0,120
metallic	39	33	0,140	35	17	*	0,011
fresh fish oil	19	23	0,593	17	8	**	0,006
acidic	9	9	1,000	8	8		1,000
earthy	8	10	0,708	8	11		0,182
rancid	1	1	0,203	1	3		0,181
TEXTURE							
soft	72	62	0,177	73	39	**	0,008
juicy	66	60	0,518	69	34	*	0,017
tender	73	69	0,457	74	52	**	0,010
mushy	24	21	0,420	23	16		0,266
sticky	34	36	0,741	27	21		0,379

ms (marginal significance, p = 0,05-0,10); * (p < 0,05); ** (p < 0,01).

A marginal difference was seen between groups for colour values a and b (Table 5). Group *Initial fish* had a higher L-value than slaughtered fish at end of *Trial 2* (p = 0,050).

Table 5. Colour measurements and p-values for difference between groups.

	Initial	Trial 1	Trial 2	p-value
L	43 **a**	40	40 **b**	0,050
a	13	11	14	0,095
b	19	15	17	0,078

Group *Initial fish* had less cooking yield than slaughtered fish at end of both Trial groups. Cooking yield for group *Initial fish* was on average 88% but 91% for end of both Trial groups. (p = 0,014). After cooking, the appearance of some samples was unusual, deformed and shrunken (Figure 4).

Figure 4. Arctic char samples from end of Trial 2, after cooking for 8 minutes and cooling for 30 minutes at room temperature (cooking yield). Counting from left to right, very deformed samples are: nr. 1, 4 and 6. Slightly deformed samples are: nr. 2, 3, 8 and 9. Samples nr. 5 and 7 are normal in appearance.

It is likely that the stress that the fish encountered in *Trial 2* due to the high bio-load, affected the flavour, odour, appearance and the texture quality of the surviving fish. The flavour and odour of the cooked samples was less characteristic than that of the Arctic char at the start of trial and the texture became firmer and drier. It is likely that the deforming on cooking, the high cooking yield and white precipitations may also have been caused by stress related symptoms. It has been reported that the muscle of stressed fish can have reduced water-holding capacity and increased incidence of gaping as well of denaturation of muscle proteins and liquid loss due to low pH levels (Daskalova, 2019). In our trials water-holding capacity was not measured nor the muscle pH; the low moisture in the fish at the end of the trial (Table 3) and the white precipitation may indicate liquid loss as well as the high cooking yield and deformed muscle on cooking (Figure 4).

The same reasoning may indicate that the Arctic char in *Trial 1* also experienced some stress related symptoms, e.g. the higher incidence of gaping, high cooking yield and somewhat less soft and juicy texture compared with that of the *Initial fish,* but at a much lower degree than that seen in *Trial 2*.

4. Conclusion

Arctic char could be held at a density of 80 kg/m^3 at 4°C for 8 days in a RAS system where the pH was controlled and the accumulated ammonia removed, without adverse effects on mortality. Moreover, no differences were found in the sensory quality (flavour, odour, appearance and texture) of the stored fish compared with fish before it was placed in the RAS system. The stored fish had however a higher incidence of gaping, higher cooking yield and marginally lighter colour than that of fish before placing in the system.

A density of 135-145 kg/m^3 Arctic char in the RAS storage and holding system led to high mortality of the fish. Moreover, on slaughter the surviving fish had adverse sensory quality as indicated by loss of characteristic flavour and odour as well as firmer, drier and tougher texture. The fish had more incidence of gaping, a high cooking yield and showed evidence of deformation on cooking.

5. Acknowledgements

The authors thank Marvin I. Einarsson, mechanical engineer, Ásbjörn Jónsson, food scientist, Giang Nguyen, Ph.D. student, and Davíð Gíslason, biologist for scientific advice and technical help in carrying out the trials.

6. References

AOAC (1995). Salt (Chlorine as Sodium Chloride) in Seafood: Potentiometric Method. Sec. 35.1.19, Method 976.18. In Official Methods of Analysis of AOAC International, 16th ed., P. Cunniff (Ed.), 8. AOAC International, Gaithersburg, MD.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.

Daskalova, A. (2019). Farmed fish welfare: Stress, post-mortem muscle metabolism, and stress-related meat quality changes. A review. Int Aquat Res 11, 113-124.

Erikson, U. (2009). Guide for evaluating fillet texture in Atlantic salmon. Fishery and Aquaculture Industry Research Fund (FHF): SINTEF Fishery and Aquaculture AS

H.T. Lawless, H. Heymann. 2010. Sensory evaluation of food, Principles and practices. Springer Science + Business Media. P. 362-366.

ISO (1997). Determination of nitrogen content and calculation of crude protein content (5983). Geneva, Switzerland: The International Organization for Standardization

ISO (1999). Determination of moisture and other volatile matter content (6496). Geneva, Switzerland, International Organization for Standardization

ISO (2014). Sensory analysis - General guidance for the selection, training and monitoring of selected assessors and expert sensory assessors (8586). Brussels, Belgium: European committee for standardization.

NMKL (2006). Aerobic count and specific spoilage organisms in fish and fish products. NMKL 184.

Summerfelt, S.T., Wilton, G., Roberts, D., Rimmer, T. and Fonkaldsrud, K (2004). Developments in recirculating systems for Arctic char culture in North America. Aquacult. Eng. 30, 31-70.

7. Appendix

Matís ohf.
Vínlandsleið 12
113 Reykjavík

Staður, 10. september 2019
Tilvísun: 19081003

Subject: Permit for animal experiment no. 2019-05-09, valid until 11/30/2019

Your application, dated 29.08.2019, for a permit for the "Transportation of live seafood" experiment was received by the National Food Authority on the same day The Application has been reviewed by the authority. According to Art. Act no. 55/2013 on animal welfare, the Authority must obtain the comments of the Animal Welfare Council. The application was reviewed at the councils meeting of 09/10/2019 and its comments are now available.

The Authority considers that the experiment is in accordance with the provisions of Regulation no. 460/2017 (IS) on the protection of animals used for scientific purposes and hereby authorizes the requested study. The condition for granting the permit is that the applicant or guarantor: must ensure that the experiment is carried out in accordance with the protocol (protocol) accompanying the application, but according to Art. Article 36 according of the regulation, the Authority monitors compliance with its provisions and data on which the permit is based.

The Authority points out that in accordance with the provisions of Art. 35 of the aforementioned regulation, the licensee shall submit a report, **before March 1, each year**, to the Icelandic Food and veterinary Authority on the experiments it made in the previous year.

Cases of violation of the regulation are dealt with in accordance with the provisions of Chapter X of Act no. 55/2013 (IS) on animal welfare. In accordance to Art. 5 of the regulation; an experiment may not be modified without obtaining a new permit and also that the Authority may temporarily suspend or revoke an a permit if the licensee violates the provisions of the regulation or the conditions of the permit.

With reference to Article 33 Act no. 55/2013 on animal welfare, the Food Authority may charge a fee for the processing of animal testing permits. Fee will be sent by mail from the Authority for this fee. Requesting a speedy treatment may involve additional costs.

Respectfully
on behalf of MAST

Sigurjón Njarðarson, representative of Iceland's Chief Veterinary

Sigurjón N.

Austurvegur 64 • 800 Selfoss • Iceland • Tel + 354 530 4800 • www.mast.is • mast@mast.is

Figure 6. MAST permission for MATIS to carry out live holding trials on Arctic char.

Experimental/ Treatment Group: Arctic char holding trials, September 2019

Date: **Time:** **AEC Number:** **Name of person scoring:**

Name of Supervisor/Chief Investigator: Giang Nguyen **Contact Telephone Number:** **After Hours:**

Indicators	Scoring of independent variables:	Individual score for affected fish in group*					
Date							
General Health Swimming	0. normal 1. intermittent loss of equilibrium 2. frequent loss of equilibrium 3. complete loss of equilibrium						
Body Score (Estimated)	0. normal 1. loss of 10-15% BW 2. loss of 15-20% BW 3. loss of >20% BW						
Abnormal abdominal muscle tone	0. normal 1. mild 2. moderate 3. severe						
Abdominal Distension	0. normal 1. mild 2. moderate 3. severe						
Behaviour	0. normal 1 - 3. all fish at surface gasping for air						
Total Score							

For Total Scores

0 = normal: no action

*** *A score of 3 in any one category: euthanase*

1-4 = moderate changes: should be monitored daily

5-8 = significant changes: monitor twice daily

>8 = euthanase

* This score-sheet is to be used following the identification of individual abnormalities within single aquaria

Signature of person scoring:

.....

Figure 7. Score sheet for scoring endpoints in Arctic char

Table 6. Scale for gaping in fillets. Reference photos see Figure 8.

score	description	photo
0	no gaping	0
1	A few small cracks	1
2	A few small cracks in less than 10% of the fillet or one bigger crack	2
3	Many small cracks in 10 to 20% of the fillet or two to three bigger cracks	2-3
4	Slight gaping in 20 to 30 % of the fillet or four to five bigger cracks	3
5	Some gaping, many big cracks or less gaping in 30 to 50% of the fillet	4
6	Very much gaping, many big cracks and gaping in 50 to 100% of the fillet	5

Gaping

Gaping is evaluated in three zones on the fillet: loin, belly and tail. To provoke gaping, the fillet shall be exerted by breaking it with a certain force. Start in the neck region and fold the loin sideways, as shown on the photo, and then continue along the fillet backwards until you reach the tail. Repeat in the same manner for the belly then evaluate the degree of gaping by comparing the fillet with the photos.

Score table for evaluation of gaping:

Score 0

Score 1

Score 2

Score 3

Score 4

Score 5

Figure 8. Reference photos from "Guide for evaluating fillet texture in Atlantic Salmon" (Erikson, 2009).