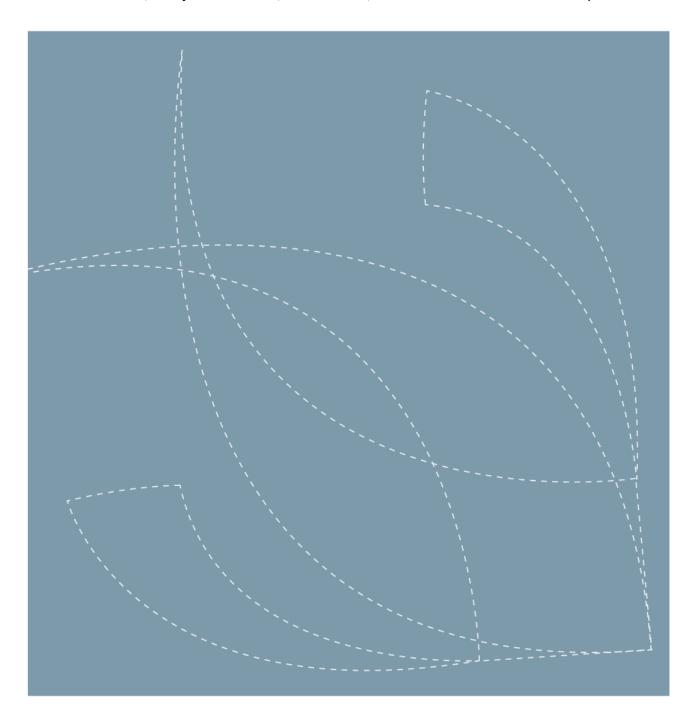


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# Cold storage of salmon and rainbow trout

Quality and shelf life

Stein Harris Olsen, Torbjørn Tobiassen, Karsten Heia, Anette Hustad and Sten Siikavuopio





Nofima is a food research institute that engages in research and development for the aquaculture, fisheries and food industries.

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#### Summary/recommendations:

For many years the position of salmon farming has become stronger on the global market, while Norwegian rainbow trout farming has declined and currently accounts for about 5% of total salmon and trout production.

Relatively few comparative studies have been conducted with regard to possible quality differences between salmon and rainbow trout under similar environmental conditions. A storage experiment was therefore set up for salmon and rainbow trout which was conducted under equal environmental conditions with respect to shelf life and quality. Our results show that salmon performed slightly less well than rainbow trout in terms of muscle quality and shelf life. After 14 days in cold storage the residual shelf life of rainbow trout was assessed as being approx. 4 days while for salmon it was 0 days. In addition, the force required for pulling pin bones from fillets of rainbow trout was about twice as much as that required for pulling pin bones from salmon fillets. We must therefore reject our initial hypothesis that there is no difference between salmon and trout bred under similar environmental conditions. Both salmon and trout had feed regimes that had been optimised for the species. It is not certain how much the various feed regimes affect quality and there may also be other underlying causes of these differences. However, new comparative experiments should be set up under which all the fish in the experiment would receive identical feed regimes. This is designed to identify whether or not the different feed regimes are the cause of the surprising findings that we have seen in this experiment.

#### Oppsummering/anbefalinger:

Oppdrett av laks har over mange år styrket sin posisjon inn mot et globalt marked, samtidig har norsk regnbueørretoppdrett gått tilbake og utgjør i dag cirka 5 % av den samlede laks- og ørretproduksjonen.

Det er gjort relativt få komparative studier med tanke på mulige kvalitetsforskjeller mellom laks og regnbueørret under like miljøbetingelser. Det ble derfor satt opp et lagringsforsøk på laks og regnbueørret kjørt under like miljøbetingelser med tanke på holdbarhet og kvalitet. Våre resultater viser at laks kom noe dårligere ut sammenliknet med regnbueørret med tanke på muskelkvalitet og holdbarhet. Etter 14 dager kjølelagring, var restholdbarheten for regnbueørret vurdert til cirka 4 dager og for laksen var det 0 dager restholdbarhet. I tillegg var trekkraften for å få ut pinnebein fra filet av regnbueørret cirka dobbelt så høy, sammenlignet med trekkraften for å få ut pinnebein fra laksefiletene. Vi må derfor forkaste vår utgangshypotese om at det ikke er forskjell mellom laks og ørret oppdrettet under like miljøbetingelser. Både laks og ørret fikk fôringsregimer optimalisert til art. Hvor mye de ulike fôringsregimene påvirker kvalitet er usikkert, i tillegg kan det være andre bakenforliggende årsaker til forskjellene. Uansett, det bør settes opp nye komparative forsøk hvor alle fiskene i forsøket får identiske fôringsregimer. Dette for å kartlegge om de ulike fôringsregimene er årsaken til de overraskende funnene som vi har vist i dette forsøket.

## **Contents**

1	Introd	duction	1
2	Mate	rials and method	2
	2.1.1	Fish	2
	2.1.2	Sensory evaluation (QIM)	
	2.1.3		
	2.1.4	Bone pulling from rainbow trout and salmon fillets	3
	2.1.5	Statistics	
3	Result	ts and discussion	5
	3.1	Sensory evaluation (QIM)	5
		Instrumental measurement of muscle colour	
	3.3 E	Bone pulling from rainbow trout and salmon fillets	9
4	Concl	usion	11
5	Refer	ences	12

## 1 Introduction

For many years the position of the Norwegian salmon (*Salmo salar*) industry has become stronger on the global market. At the same time, there has been a decline in Norwegian rainbow trout (*Oncorhynchus mykiss* farming which currently accounts for about 5% of total salmon and trout exports (SSB, 2016). Future growth and a positive reputation for the industry require stable production and good quality products. As regards fish muscle, the colour, firmness and absence of fillet gaping are some of the most important quality criteria. Norwegian rainbow trout has an advantage because when compared to salmon its meat acquires a better red colour, even when it starts to become sexually mature (Siikavuopio *et al.*, 2016; 2017). It is well known that annual variations in the environment, climate, state of health, growth, feed composition and feed regime can affect the muscle quality of salmon. Examples are soft fillets, fillet gaping and varying colour and appearance (Mørkøre, 2008; Mørkøre *et al.*, 2010; Mørkøre, 2012; Mørkøre *et al.*, 2014; Sissener *et al.*, 2016).

The aim of this project is to produce rainbow trout where the focus is on high-quality roe combined with good quality fish. Compared to ordinary fish production this type of production will present different challenges and opportunities. It currently takes over 2 years in the sea to produce mature rainbow trout. This is associated with risks such as repeated delousing and handling, especially in respect of large fish. It is also well known that sexual maturation leads to changes in the texture and that the percentage of fish with reduced muscle colour and discoloured fillets increases towards the end of the maturation phase. The project follows a normal selection of fish over time; before and during final maturation, based on the quality of the roe and fillets (Siikavuopio *et al.*, 2017). The results of previous experiments show that rainbow trout muscle changed towards final maturation. This includes loss of muscle colour and softer muscle consistency, compared to immature rainbow trout. At the same time when comparing it with salmon, we observe that both the colour and texture of muscle in sexually mature rainbow trout was similar to that of superior salmon, stored under the same conditions.

According to the plan, a new selection of slaughter-ready rainbow trout would be followed up in 2017, taking into account muscle quality and large-scale roe production. As a result of major challenges involving sea lice and disease, Svanøy Havbruk was ordered by the Norwegian Food Safety Authority to slaughter all its spawner fish during the summer of 2017. As a result, Nofima was unable to carry out planned sample extraction, with a view to following up the status of mature rainbow trout prior to their final maturation in 2017. The results achieved in 2015 and 2016 indicated that the muscle of rainbow trout is firmer in structure and can withstand cold storage better than salmon. In the absence of roe and following consultation with the client, we decided to undertake a more systematic study on this issue. Immature rainbow trout and salmon were slaughtered from the same locality and at the same time at Svanøy Havbruk.

Our initial hypothesis was that salmon and rainbow trout kept under similar farming conditions in the sea have the same product characteristics in terms of shelf life and quality. In order to investigate this, a comparative cold storage experiment was set up with equally large rainbow trout and salmon, over a trial period of 14 days.

## 2 Materials and method

#### 2.1.1 Fish

Salmon smolt (70–80 g) (Elite from AquaGen) and rainbow trout smolt (All-Female from AquaGen) were placed in the sea during the autumn of 2016, at Svanøy Havbruk's farm in Sogn og Fjordane. The average sea temperature between the time they were placed in the sea and until they were slaughtered was about 10 °C. The rainbow trout were mainly fed Opal Silva 200 40A and 60A from EWOS during their first year in the sea, and then with Premium Trout 600 and 2500 from Skretting. For a few weeks during the final six months prior to slaughter, PF Biofeed Aqua Forte Trout from Polarfeed and Aller Active BF from Aller Aqua were also used.

The salmon were also fed a similar diet to that of the rainbow trout, i.e. varied feed and feed suppliers. During their first six months in the sea, the salmon received feed produced by EWOS. Most of the feed used during this period was Opal 200 AQSG 40A and SG 500AQ 50A. From the summer of 2017 until slaughtering in January 2018, the salmon received feed from Polarfeed (PF Biofeed Aqua Forte 300 and 800) and from Skretting (Premium Trout 2500, Select SG V 2500 and Protect Gill 2500).

In January 2018, immature rainbow trout and salmon were slaughtered, placed in ice in polystyrene boxes and sent from Svanøy Havbruk to Nofima. At Nofima, the rainbow trout  $(2.8 \pm 0.1 \text{ kg}; n=10)$  and salmon  $(3.2 \pm 1.0 \text{ kg}; n=10)$  were placed in cold storage  $(0-1^{\circ}\text{C})$ . Eight days after slaughter, the rainbow trout (n=5) and salmon (n=5) were taken out for sensory quality evaluation (QIM). The remainder were kept in cold rooms for a further 6 days before a new sensory quality evaluation was carried out. Following sensory evaluation, all the fish were filleted, before they were evaluated by using diffuse reflectance spectroscopy. In order to compare the strength of the texture and connective tissue of the fillets, the pulling force required in order to remove the pin bones from the fillets was also measured.

## 2.1.2 Sensory evaluation (QIM)

QIM (Quality Index Method) is a standardised method used for sensory determination of the quality of whole fish stored in ice. It is based on simple evaluation of the appearance and smell of the fish. The score obtained (QIM score) does not indicate how long the fish has been stored for, but how long it can remain in cold storage before it is considered to be unfit for human consumption.

The QIM evaluation that has been developed for farmed salmon was used in this experiment (Sveinsdottir *et al.*, 2003) in order to assess the shelf life of both rainbow trout and salmon. The method has previously been used by others for evaluating the shelf life of rainbow trout (Erikson *et al.*, 2017). The smell and appearance of the fish were assessed by 2 trained individuals and a QIM score of 15 indicates the maximum shelf life of salmon in cold storage.

#### 2.1.3 Instrumental measurement of muscle colour

After filleting, the colour of the muscle was measured instrumentally by using diffuse reflectance spectroscopy. This is an objective method used for measuring the colour of fish muscle. The instrument reads fillets at a rate of 50 cm per second (Fig. 1). The instrument has the capacity to take photos of over 216 colour channels that include both visible and infrared light. Reflectivity is a technical expression which indicates how much light a surface absorbs and then reflects back to the measuring

instrument (Heia *et al.*, 2012). The colour scores for each fillet were also assessed by trained personnel using a SalmoFan<sup>TM</sup> ruler (DSM, Switzerland).



Photo 1 Instrumental reading of a rainbow trout fillet at a rate of 50 cm per second

## 2.1.4 Bone pulling from rainbow trout and salmon fillets

In each fillet, the pulling force was measured for 4–5 pin bones, located in the same area on all fillets, using a method described by *Akse et al.* (2011). A Lutron FG-5000A electronic force gauge (Lutron Electronic Enterprise Co., Ltd, Taiwan) was used for measuring the pulling power in grams. The instrument was attached to a clip which was then attached to the end of each pin bone (Fig. 2). The measurement of the pulling force to remove the bones was carried out immediately after the instrumental colour measurement, on day 8 and day 14 after slaughtering. The purpose was to see if the pulling force changed during the course of storage, as well as to see whether or not there were any differences in the pulling forces required for salmon and trout. This is a measure of how well the bones are anchored in the muscle during storage, as well as an indicating of the possible differences in the strength of the connective tissues and muscles of salmon and rainbow trout.



Photo 2 The photo on the left shows a clip being attached to the end of a pin bone in a salmon fillet. The photo on the right shows the actual measurement set up.

## 2.1.5 Statistics

Microsoft Excel was used for data processing and statistical analysis of the data. In order to test whether or not there were any significant differences between the fish before and after live storage, a two-way T-Test was performed. The significance was set to p<0.05. The p-value is a number between 0 and 1 and shows the likelihood of obtaining an equal test result. The lower the p-value, the more likely it is that there will be differences in the values relating to salmon and rainbow trout. The values in the document are average ± standard deviations, unless otherwise specified.

## 3 Results and discussion

## 3.1 Sensory evaluation (QIM)

Based on the results, the shelf life of the salmon is relatively short (approximately 14–15 days) compared to the QIM standard, which indicates a shelf life of 19–20 days for farmed Atlantic salmon stored in ice (Sveinsdottir *et al.*, 2003). A similarly short shelf life for farmed salmon has been reported earlier (Tobiassen *et al.*, 2013). The salmon achieved a QIM score of 6.4 after 8 days of cold storage and a QIM score of 15.0 after 14 days. Based on the QIM standard, a score of between 6 and 7 corresponds to a residual shelf life of between 11 and 13 days. A QIM score of 15 indicates 0 days of residual shelf life, i.e. the fish should not be sold due to its different smell, colour or consistency.

As for the rainbow trout, it performed better than salmon as early as day 8. After 14 days on ice, the rainbow trout achieved a QIM score of 12.0. According to the QIM standard, this corresponds to a residual shelf life of another 4 days. In other words, the total shelf life of this batch of rainbow trout will probably extend to 18–19 days, which is more in accordance with the QIM 2003 standard. In the salmon, there was a loss of flexibility in the muscle. The development of an acidic fermented odour of the skin, the abdominal cavity and the gills also contributed towards a reduction in the overall QIM score (see Table 1).

The reasons for the short shelf life of the salmon, compared to the rainbow trout, are complex, and one cannot rule out that several biological, production and environmental factors may be involved, particularly when bearing in mind the major production changes that have taken place within the salmon industry over the past 20 years. These include more rapid fish growth between the fry and slaughtering stages, as well as changes in the feed recipe. In our case, these fish groups were given different diets at the end, which can also help to explain the differences in colouring, etc. Large volumes of slaughtered fish also place greater demands on how the slaughterhouses deal with transport, production flow, animal welfare requirements and adequate bleeding and cooling. A larger comparative study that spans several production cycles should be carried out in order to shed light on possible reasons as to why the salmon performed slightly worse than shown in the QIM standard.

Table 1	Overview of QIM s	cores for salmon and	d rainbow trout s	stored for 8	3 and 14 days.
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QIM analysis		9	Skin		Eye	es		Gills		Abdominal	cavity	Total
Salmon	Colour	Mucus	Odour	Texture	Pupils	Shape	Colour	Mucus	Odour	Blood colour	Odour	QIM score
Day 8 Average (n=5)	0.2	0.0	1.0	1.2	1.0	1.0	0.2	0.2	1.0	0.0	0.6	6.4
Day 14 Average (n=5)	1.0	0.8	1.2	1.8	2.0	1.6	1.2	1.0	2.0	0.6	1.8	15.0
QIM analysis		9	Skin		Eye	es		Gills		Abdominal	cavity	Total
Rainbow trout	Colour	Mucus	Odour	Texture	Pupils	Shape	Colour	Mucus	Odour	Blood colour	Odour	QIM score
Day 8 Average (n=5)	0.0	0.0	0.4	1.0	1.0	1.0	0.2	0.0	1.0	0.0	0.0	4.6
Day 14 Average (n=5)	1.0	0.4	0.4	1.4	1.6	1.6	1.2	1.0	1.4	0.6	1.4	12.0



Photo 3 Salmon ready for QIM analysis. QIM analysis includes assessment of the skin, eyes, gills and abdominal cavity in fish, taking into account changes in smell, colour, shape and consistency.



Photo 4 After the QIM analysis, the fish were filleted and trimmed by hand, before undertaking colour measurements and pulling the pin bones.

#### 3.2 Instrumental measurement of muscle colour

As expected, the rainbow trout fillets were significantly redder than the salmon fillets. The rainbow trout was rated as having a SalmoFan score of approx. 32–33, and the salmon was rated at 25–26. Storing salmon results in a natural degradation of the colour pigments in the muscle and this gradually leads to a paler look during the course of storage. This process is somewhat delayed during cold storage in ice. This can be seen, for example, in the high SalmoFan scores achieved on day 14. As regards the SalmoFan scores for the salmon, this is a score that corresponds to very well-coloured salmon muscle. Measurement of the SalmoFan scores was also carried out using diffuse reflectance spectroscopy (Figs. 6 and 7). This is an instrumental, objective, rapid method used for measuring the colour of fillets. Instrumental colour measurements were only taken for fish fillets that had been stored for 8 days, and as shown in Table 2, these measurements correspond well with the visually assessed SalmoFan score.

Table 2 The instrumental colour measurements corresponded well with the visually assessed SalmoFan score.

Species	Method	SalmoFan Day 8	SalmoFan Day 14
Calman	Visually assessed	26–27	25–26
Salmon	Instrumental reading	26	
Daimhanntannt	Visually assessed	33–34	32–33
Rainbow trout	Instrumental reading	33	

In 2 out of 10 salmon, varying degrees of paleness were found in the muscle along their backs (Photo 5). There could be several reasons for the incorrect pigmentation and differing colours, e.g. rapid growth, feed source or low sea temperatures can inhibit or reduce the uptake of pigments. It is also known that pancreatic disease (PD) can lead to discoloured or pale areas on salmon fillets (Mørkøre, 2012).



Photo 5 Shows incorrect pigmentation of salmon fillets. In this sample, 2 out of 10 fish fillets had pale areas, as shown in the photo.

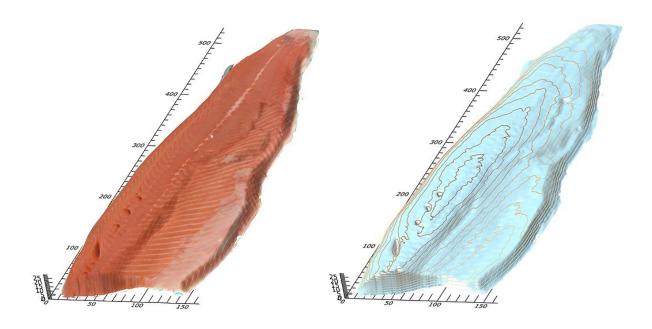


Photo 6 Shows a 3-D scanned salmon fillet (right). The colour of the same fillet (left) was measured by using diffuse reflectance spectroscopy, and it was then placed over the 3-D model. The advantage of this is that the instruments can record fillet gaping, and the thickness and colour of each fillet. As shown in the photo, the 4 gaps in the front part of the loin can easily be seen. The SalmoFan values were measured within a given range on the fillets (circled).

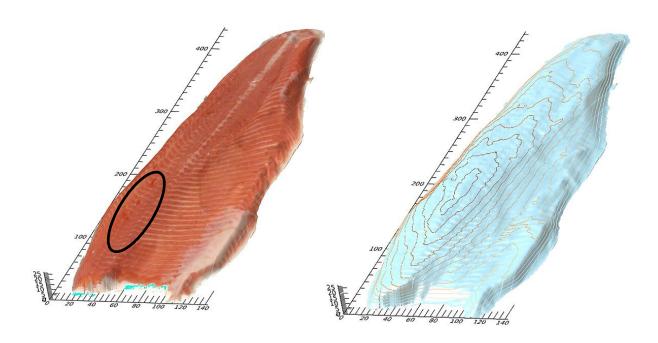


Photo 7 Shows a 3-D scanned rainbow trout fillet (right). The colour of the same fillet (left) was measured in a similar way by using diffuse reflectance spectroscopy and then placed over the 3-D model. None of the rainbow trout fillets showed any signs of fillet gaping 8 days after slaughtering. The SalmoFan values were measured within a given range on the fillets (circled).

## 3.3 Bone pulling from rainbow trout and salmon fillets

The pulling force required to remove pin bones from the salmon fillets was significantly (p<0.05) lower, compared to the pulling force needed to remove pin bones from rainbow trout fillets. In some rainbow trout fillets the pin bones were so firmly attached to the muscle that the muscle segments (myotomes) around the bones ruptured. The same force was measured when pulling pin bones from the trout fillets, both 8 days and 14 days after slaughtering. As regards the salmon fillets, the pin bones were much more loosely attached to the fillets. Most of the bones released the muscle easily without damaging the fillet itself. In addition, a significant (p<0.05) reduction in the pulling force was measured between day 8 and day 14. In previous experiments on pulling the bones from salmon fillets, it was found that a force of around 200–300 grams was required in order to pull the bones out of the salmon fillets that had been stored for 6 days in ice (Esaiassen & Sørensen, 1996). This is consistent with what we found in our experiments, indicating that the strength of the connective tissue in which the bones are attached has not changed significantly during the last 20 years.

The results of our experiments suggest that the bones of rainbow trout are better anchored in the connective tissue than is the case for salmon. Based on these results, no changes in the pulling force were measured between 8 and 14 days after slaughtering for rainbow trout. Stronger connective tissue membranes in the rainbow trout appeared more clearly on the fillets than those we could see on the salmon fillets (Photo 8). The rainbow trout fillets still had good flexibility and had a firmer texture, compared to the salmon, after 14 days of cold storage. The salmon fillets were somewhat softer and the tail piece in particular lacked flexibility. This part of the fillet had a dough-like consistency and finger marks remained behind in the muscle, even when slight finger pressure was applied. Soft texture and fillet gaping have been a challenge for the salmon industry for several years. The challenges posed by soft texture are complex and involve many different factors, including focus on locality, growth patterns, feed recipes, seasonal variations and disease (Mørkøre, 2012; Mørkøre et al., 2013)

Table 3 Measurement of the pulling force required to remove the pin bones (n=4) from rainbow trout (n=5) and salmon (n=5) fillets, measured 8 and 14 days after slaughtering. The letters a) and b) indicate significant (p<0.05) differences between day 8 and day 14. The asterisk \*) indicates significant (p<0.05) differences between salmon and rainbow trout.

Characations	Salmon	Rainbow trout
Storage time	Bone pulling fo	orce (grams)
8 days	319 <sup>a)</sup> ± 86	480*) ± 122
14 days	256 <sup>b)</sup> ± 100	487*) ± 120



Photo 8 Rainbow trout fillet on the left and salmon fillet on the right. There is a clear difference in muscle colour. The white connective tissue (myosepta) between the muscle segments (myotomes) can be seen better in the rainbow trout than in the salmon. In the salmon, the myosepta are not shown in the tail (circled in the photo). There is also a dough-like consistency in the muscle behind the tail, compared to the rainbow trout fillet.

## 4 Conclusion

In this experiment, the salmon performed worse than the trout in terms of muscle quality and shelf life. We must therefore reject our initial hypothesis that there is no difference between salmon and trout bred under similar environmental conditions. Both the salmon and trout had feed regimes that had been optimised for species. How much the different feed regimes affect the quality is uncertain. New experiments should be set up in which the experimental fish would receive identical feed regimes in order to observe the extent to which the different feed regimes are the cause of the surprising findings in this experiment. A further study should also be more comprehensive in terms of the number of fish in each extraction. In addition, extractions must be made at different times throughout the year in order to include seasonal variations. Such an experiment may also be able to reveal whether or not there may be other underlying causes behind the differences between rainbow trout and salmon.

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