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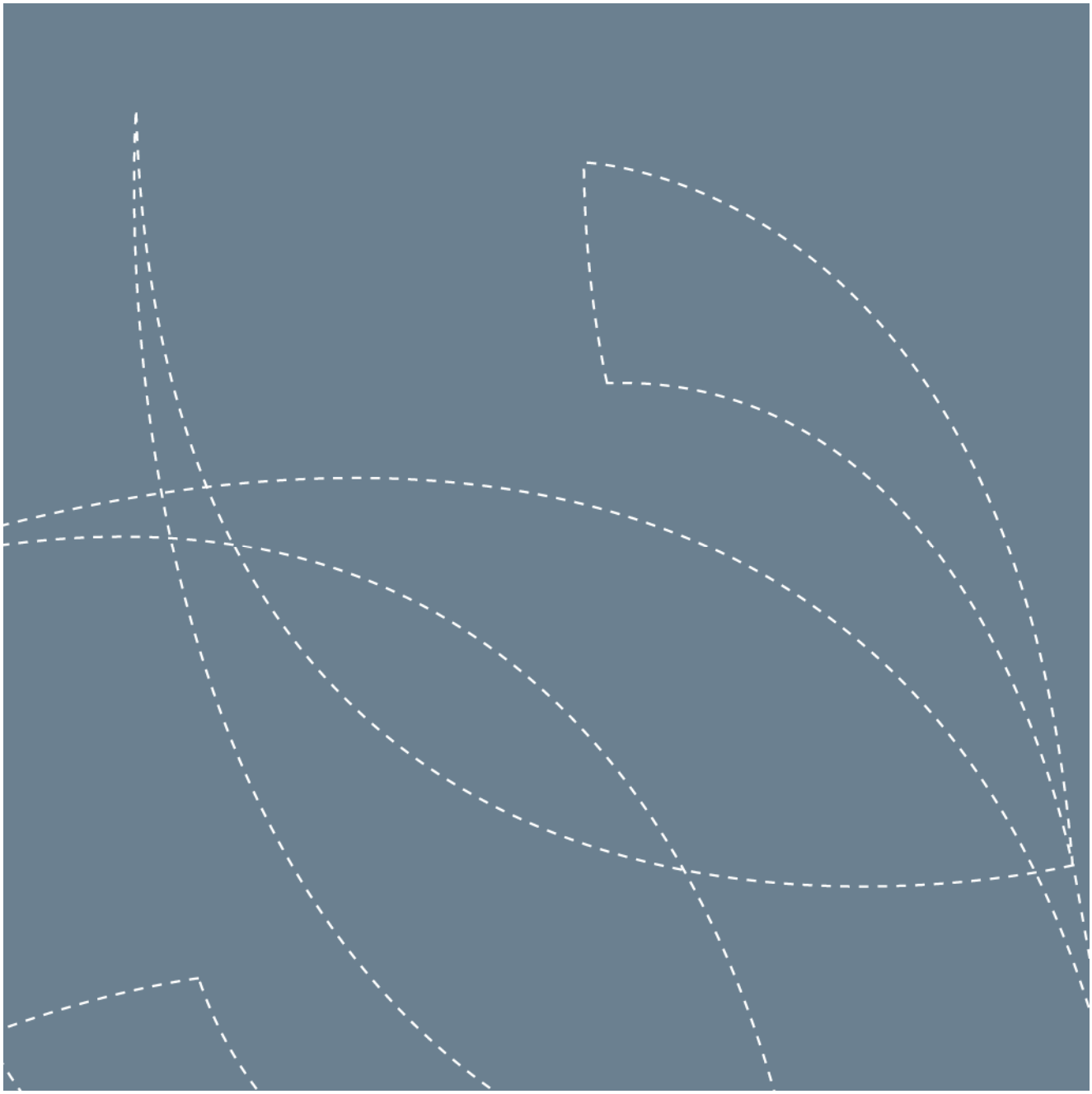
Feed utilisation in Atlantic salmon kept at fluctuating oxygen saturation

A CREATE project

Turid Synnøve Aas, Mette Remen, Frode Oppedal, Tor Johannes Hjertnes



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Summary:

Atlantic salmon (initial weight 383 g) was kept at either stable oxygen saturation (>80%, control) or exposed to four daily drops in oxygen saturation to 70, 60 or 50% in a 68-days experiment. The fish was fed twice daily during normoxic (>80% oxygen saturation) periods. At termination of the trial, the feed intake and growth was significantly correlated to oxygen saturation during the hypoxic periods, and the SGR ranged from 1.16 (70% group) to 0.99 (50% group), and the range of TGC was from 1.97 (control and 70% groups) to 1.67 (50% group). Treatment did not significantly affect FER, apparent digestibility of macronutrients or retention of these. The growth was suboptimal in the trial, probably due to sexual maturation that was observed.

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1 Introduction

In intensive fish farming, a sufficient oxygen supply is critical for fish growth, health and welfare. At present, sea pens with 160 m circumference are commonly used in Norwegian fish farming, stocked with several hundred tons of salmon. Large pens, high stocking densities, high water temperatures and periods of restricted water exchange challenge the oxygen supply. In particular, periods with low water exchange due to the turn of tidal currents, combined with high water temperature, can lead to critically low levels of oxygen (Johansson *et al.*, 2006;Johansson *et al.*, 2007;Vigen, 2008;Oppedal *et al.*, 2010).

Fluctuating oxygen levels are more common in aquatic than in terrestrial environment, and fishes, some species more than others, are to a certain degree adapted to cope with hypoxic environment through behaviour and/or metabolic adjustments (Wells *et al.*, 1989;Waller *et al.*, 1997;Ritola *et al.*, 2002;Zhang *et al.*, 2009;Ludsin *et al.*, 2009). Atlantic salmon in the oceanic phase does not normally inhabit hypoxic areas and rely on aerobic metabolism for rapid and sustained swimming. Salmonids are considered sensitive to restricted oxygen supply (Bickler & Buck, 2007).

Oxygen is the terminal electron acceptor in aerobic cellular metabolism, and when oxygen is depleted, anaerobic metabolism is elevated compared to normoxic situations (Mohamed & Kutty, 1983). Increased plasma glucose and lactate and decreased plasma concentration of free fatty acids are found in fish exposed to hypoxia (van Raaij *et al.*, 1996;Person-Le Ruyet *et al.*, 2003). Martínez et al. (2006) showed that the activity of several enzymes involved in energy metabolism was affected by hypoxia in Gulf killifish (*Fundulus grandis*), and that the effect of hypoxia on these enzymes was tissue-specific. The specific dynamic action, which is the increased metabolic rate following a meal, is also affected by hypoxia in teleost fish (Jordan & Steffensen, 2007). When exposed to oxygen saturations below a critical level, this leads to stress, and if sustained, a loss of equilibrium and eventually death (van Raaij *et al.*, 1996;Lays *et al.*, 2009).

Suboptimal oxygen saturations can effect feed intake, growth and feed utilisation (Chabot & Dutil, 1999;Pichavant *et al.*, 2000;Person-Le Ruyet *et al.*, 2003;Chabot & Claireaux, 2008), which in turn will have economic consequences for the fish farmer. In order to avoid negative effects of hypoxia, limits for suboptimal and critical oxygen saturations must be established. This study focuses on cyclic hypoxic periods at high temperature (16 ºC), caused by tidal fluctuations in water exchange rate, as this has been observed to be a problem in Norwegian salmon net pens in autumn.

The aim of the present study was to investigate the effect of different oxygen levels in cyclic hypoxic periods on Atlantic salmon welfare and performance. This report describes the results obtained regarding the feed utilisation. Other results from the trial are reported separately.

2 Materials and methods

2.1 Feed

The experimental feed was produced at Nofima Ingrediens (Bergen, Norway). The feed was based on high quality fish meal and fish oil. Whole ground wheat was used as a binder, and yttrium oxide (Y_2O_3) was added as an inert marker for digestibility estimation (Austreng, 1978;Austreng *et al.*, 2000). The pellet size was targeted to be 4,5 mm. The formulation and chemical composition of the feed are shown in Table 1.

Table 1 Formulation and chemical composition of the feed, given as g/kg or MJ/kg.

	Content (g/kg or MJ/kg)
Formulation:	
Fish meal [a]	515.4
Wheat gluten [b]	60.6
Fish oil [c]	230.0
Whole wheat [d]	170.0
Vitamin mix [e]	20.0
Mineral mix [f]	4.0
Yttrium oxide	0.13
Carophyll Pink (10 %)	0.44
Chemical composition:	
Dry matter	938.1
In dry matter:	
Crude lipids	314
Nitrogen	74.0
Ash	72.9
Energy	25.77

[a] Norse-LT 94, Norsildmel, Bergen, Norway
[b] Amytex 100, Tate & Lyle, Belgium
[c] NorSalmOil, Nordsildmel AL, Fyllingsdalen, Norway
[d] Hvete sammalt 0, Norgesmøllene AS, Bergen, Norway
[e] 160 mg (3000 I.E) vitamin D3, 160 mg vitamin E (Rovimix, 50%), 20 mg thiamine, 30 mg riboflavine, 25 mg pyrodoxine–HCl, 200 mg vitamin C (Rovimix Stay C, 35%), 60 mg calcium pantothenate, 1 mg biotin, 10 mg folic acid, 200 mg niacin, 0.05 mg vitamin B12 and 20 mg menadion bisulphite per kg feed.
[f] 500 mg Mg, 400 mg K, 80 mg Zn, 50 mg Fe, 10 mg Mn, and 5 mg Cu per kg feed

2.2 Fish trial

Individually tagged post smolt Atlantic salmon (*Salmo salar* L.) with mean initial weight 383 g was used in the trial. The salmon was of AquaGen strain, hatched at Matre Research Station (Institute of Marine Research). Prior to the trial, the salmon was fed commercial feed (Skretting Nutra 2 and 3, and BioMar CPK 75 and 200).

February 9th-10th 2009 approximately 1300 fish with individual Floy® were distributed to 12 fibreglass tanks with diameter 3 m, water depth approximately 0.79 m (~ 5600 L water), supplied with sea water with temperature 9 ºC, salinity 34‰, and 24 h light. The temperature was gradually increased to 16 ºC (Table 2), and the oxygen saturation was kept above 80%. Initial weight and length was registered April 22nd – 23rd.

Table 2 Water temperature prior to the salmon trial.

Date	Temp. (ºC)
09.02.2009	9
10.02.2009	10
11.02.2009	11
12.02.2009	12
10.03.2009	13
26.03.2009	14
27.03.2009	15
28.03.2009	16

During the trial the salmon was subjected to four different treatments; four times daily the oxygen level was decreased to 70, 60 or 50 % oxygen saturation for 1h 40 min (Fig. 1) or remained at 80% (control),. The treatment groups are denoted 80, 70, 60 and 50 groups, reflecting the oxygen saturation during hypoxic periods, and in this report, all oxygen levels below 80% are referred to as hypoxia. The fluctuations in oxygen level were obtained by reducing the inlet water flow, and the water speed within the tanks was maintained by pumps circulating the water. Between the hypoxic periods, the oxygen saturation was kept at 80%. The temperature was kept at 16 ºC, and the salmon was fed a fixed ration twice daily during normoxia, aiming at 20% overfeeding. Each feeding period lasting 20 minutes. The feed spill was collected and feed intake estimated as described by Helland et al. (1996). Briefly, the feed intake was estimated by collecting the feed spill and correcting for expected loss of feed, determined in tanks without fish.

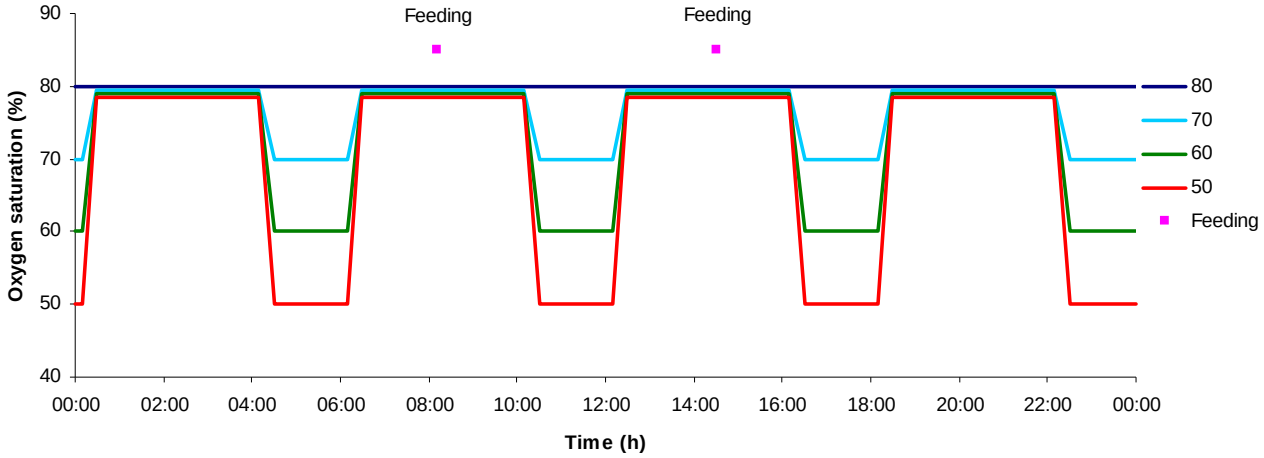

Figure 1 Time schedule for daily feeding and fluctuation in oxygen saturation.

The trial was run in triplicate, and lasted for ten weeks. The faeces was sampled after 9 weeks. The dates for hatching, transfer of the salmon to sea water and the main events of the trial are summarised in Table 3.

Table 3 Dates for hatching, transfer to sea water and main events of the salmon trial.

21.01.2008	Hatching
22.09.2008	Transfer to sea water
26.03.2009	Feeding with experimental feed started
9.-10.02.2009	Salmon placed in experimental tanks. Individually tagged with Floy tags
22.-23.04.2009	Registration of initial weight and length
24.04.2009	Start of trial and period 1. Oxygen fluctuation started.
29.04.2009	Blood samples collected (4 salmon per tank)
27.-28. 05.2009	Registration of weight and length. Biomass adjusted to 52 ±3 kg per tank. End of period 1.
29.05.2009	Start of period 2
24.06.2009	Blood samples and collection of faeces
1.-2.07.2009*	Registration of weight, length and weight of gonads. Termination of trial.

* The trial lasted longer than this and more samples were collected later, but this is not relevant for the current dataset. In this report, 1.-2.07.2009 is therefore considered as the termination of the trial.

2.3 Sampling

At start of the trial (24.04.2009), 12 fish (3 from each cage, pooled) and at termination of the trial, 10 fish from each tank (pooled by tank) were sampled for whole body analysis. One week prior to the final sampling, the fish was stripped for faeces (pooled by tank) as described by Austreng (1978). All samples were stored at -20 ºC until chemical analysis, and the faecal samples were freeze dried prior to chemical analysis. Prior to stripping, the fish was anaesthetised with Finquel (20 mg/L).

To reduce the risk of empty intestines at faecal sampling, the salmon was fed every six hours (08:10, 14:10, 20:10 and 02:10 for tank 1) the last day and night prior to faecal sampling, and the oxygen levels were reduced at 08:50, 14:50, 20:50 and 02:50. The time schedule for oxygen fluctuations and feeding for each tank during this day were set up in a staggered manner, and the sampling followed a strict corresponding time schedule (starting at 9 a.m. for tank 1), so that all tanks were sampled at exactly equal time after the last feeding and oxygen reduction.

2.4 Chemical analysis

Feed, faeces (freeze dried) and whole body were analysed for crude lipids (Soxtec HT6, Tecator, Höganäs, Sweden), N (Kjeltec Auto System, Tecator, Höganäs, Sweden), ash (550 ºC until constant weight), DM (105 ºC until constant weight) and energy (Parr 1271 Bomb calorimeter). Feed and faeces (freeze dried) were further analysed for yttrium by inductive coupled plasma mass spectroscopy (ICP-OES Optima 5300DV, at Eurofins Fôr og Mat, Moss, Norway).

2.5 Calculations

$$\text{Feed intake}\,(\text{DM}) = \text{Feed fed}\,(\text{g, DM}) - \frac{\text{Waste feed}\,(\text{g, DM})}{\text{Recovery}}$$, where

$$\text{Recovery} = \frac{\text{Feed spill}\,(\text{g, DM})}{\text{Feed used}\,(\text{g, DM})}$$ estimated according to Helland et al. (1996) by following the experimental feeding routines, but with no fish in the tanks.

$$\text{Specific growth rate}\,(\text{SGR}) = \frac{100 \cdot [\ln(\text{Final weight}) - \ln(\text{Start weight})]}{\text{Days fed}}$$

$$\text{Thermal growht coefficient}\,(\text{TGC}) = 1000 \cdot \frac{\text{Final weight}^{\frac{1}{3}} - \text{Start weight}^{\frac{1}{3}}}{\text{Sum daydegrees}}$$

$$\text{Feed efficiency ratio}\,(\text{FER}) = \frac{\text{Weight gain}\,(\text{g})}{\text{Feed intake}\,(\text{g, DM})}$$

$$\text{Feed conversion ratio}\,(\text{FCR}) = \frac{\text{Feed intake}\,(\text{g, DM})}{\text{Weight gain}\,(\text{g})}$$

$$\text{Apparent digestibility}\,(\%) = 100 \cdot \frac{a - b}{a}$$, where a is the nutrient to marker (Y_2O_3) ratio in diet and b is the nutrient to marker ratio in faeces

$$\text{Nutrient retention}\,(\%\ \text{of ingested or digested}) = \frac{100 \cdot [\text{Nutrient content at end}\,(\text{g}) - \text{Nutrient content at start}\,(\text{g})]}{\text{Nutrient ingested or digested}\,(\text{g})}$$

“Lipid retention” includes whole-body lipid from non-lipid precursors.

2.6 Statistical analysis

Tank mean was used as the statistical unit in the data from the fish trial. The trial was run in triplicate tanks in a completely randomised design, and the data are presented as mean ± S.E.M. The data were analysed with linear regression analysis, using SAS computer software (SAS 1985, SAS Institute Inc, Cary, USA), and effects were considered significant if $P<0.05$. P-values in the range 0.05-0.1 are referred to as trends.

3 Results and discussion

3.1 Feed intake and growth

The salmon in all treatment groups appeared to be at good health and there were few mortalities (4 fish) during the trial. However, at termination of the trial sexual maturation (males in particular) was observed, and this may have affected the results.

The feed intake is shown in Fig. 2. There was a significant (P<0.05) effect of oxygen level during hypoxia on the feed intake in period 1, in period 2 and in the total trial. The total feed intake per individual during the trial was 333±24, 326±7, 301±10 and 276±20 g (dry matter) in the 80, 70, 60 and 50 groups, respectively. In period 2 however, the highest feed intake was found in the salmon exposed to 70% oxygen saturation.

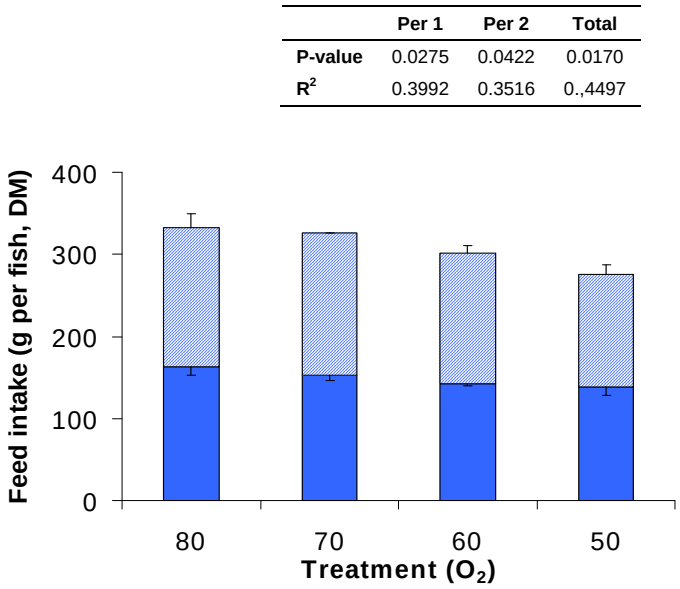

Figure 2 *Feed intake for period 1 (blue) and 2 (hatched) in Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3). Feed intake for period 1, period 2 and the total trial was significantly affected by treatment (P<0.05).*

In accordance, feed intake has been shown to be significantly reduced in European sea bass (*Dicentrarchus labrax* L.) exposed to fluctuating or low oxygen saturation (Thetmeyer *et al.*, 1999). Pichavant et al. (2000) showed that feed intake was lower in juvenile turbot kept at constant hypoxic conditions (65 and 45% O_2-saturation) than in the control group (95% O_2-saturation), although the difference declined throughout the trial.

After period 1 the body weight of the salmon tended (P<0.1) to decrease as oxygen saturation during hypoxic periods decreased (Table 4). At termination of the trial, this effect had become significant (P<0.05). Accordingly, the specific growth rate (SGR) and thermal

growth coefficient (TGC) in period 1 and in the total trial declined as oxygen saturation was lowered during the hypoxic periods (Fig. 3). This is in accordance with reduced growth observed in European sea bass kept at fluctuating oxygen saturation (Thetmeyer *et al.*, 1999).

In period 2, there was no such significant effect, and the highest SGR was found in salmon exposed to 70% oxygen saturation during the hypoxic periods, and not in the control group. This may be related to sexual maturation which was observed in the trial, and the fastest growing fish in period 1 (control group) may have reached sexual maturation first, and therefore the growth in period 2 is most affected in this group.

Table 4 *Weight of Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3).*

	80	70	60	50	P-value	R^2
Start weight (g, 24. April 2009)	387±4	375±2	386±3	386±6	0.7288	0.0126
Body weight after period 1 (g, 27.-28. May 2009)	608±19	578±9	576±3	570±14	0.0518	0.3276
Body weight after period 2 (g, 1. July 2009)	805±23	786±9	764±15	728±28	0.0109	0.4931

According to Skretting (2010), expected growth for Atlantic salmon of 400 g and 700 g at 16 ºC is 1.77 and 1.42 % per day, respectively. The highest growth was observed in period 1, when SGR ranged from 1.41±0.09 (control) to 1.22±0.06 (50% group). In period 2 the SGR ranged from 0.96±0.01 in the 70% group to 0.72±0.10 in the 50% group. The poor growth in period 2 was probably caused by the sexual maturation.

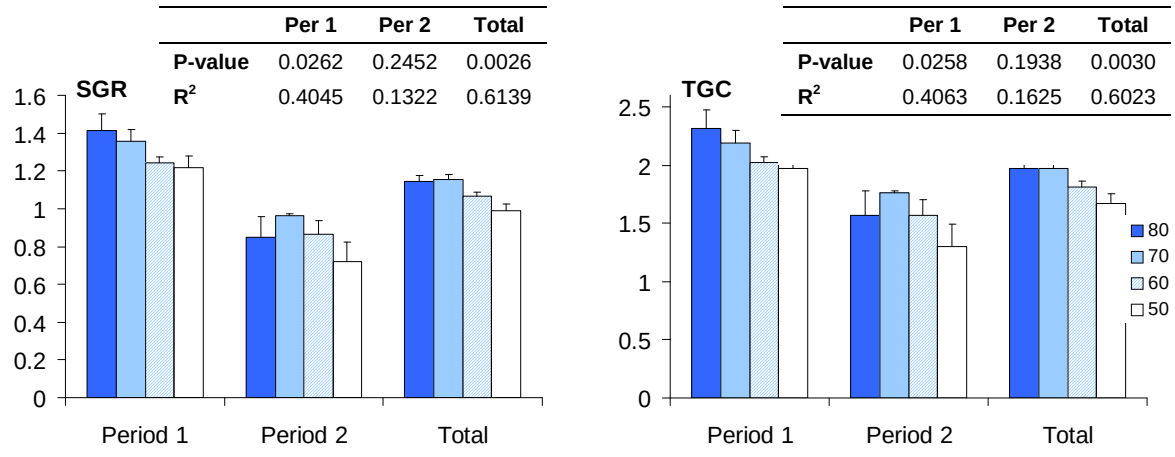

Figure 3 *SGR and TGC in Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3). SGR and TGC for period 1 and the total trial was significantly affected by treatment (P<0.05).*

3.2 FER/FCR

The feed utilisation is given both as FER and its inverse, FCR, in Table 5. There was no effect of treatment on FER (or FCR) during period 1 or period 2 separately, but given for the whole trial, the FER tended ($P<0.1$) to decrease (and FCR increase) as oxygen saturation during hypoxic periods decreased. The fish doubled the body weight during the total trial, and thus inaccuracy at weighing is of less importance for the whole trial than for each of the two periods. Besides, each of the two periods was somewhat short in time for measuring feed utilisation. Thus, the FER (and FCR) data for the total trial is most interesting.

The highest values for FER were found in the 70% group. This may, at least in part, be explained by the sexual maturation that occurred during the trial, since the largest fish, which was the control group, may have started the sexual maturation first.

At low feed intake, feed utilisation may be affected. Einen et al. (1999) showed that in Atlantic salmon fed 50-75% feed rations, FER was significantly lower than in salmon fed *ad libitum* (100% ration). In the present trial, the effect of treatment on feed intake was small, and FER was not significantly affected. Accordingly, Thetmeyer et al. (1999) found no difference in FER in European sea bass kept at normoxic, hypoxic or fluctuating oxygen saturation, and concluded that growth reflected feed intake. However, the fish with the lowest feed intake had correspondingly reduced growth, and although FER was not significantly reduced in the present trial, the fish with low feed intake will need more time to achieve the intended body weight. Since keeping the fish represents cost, it is of economic importance for the fish farmer to maintain a stable, normoxic environment. Furthermore, the FER for the total present trial ranged from 1.25 (70% group) to 1.19 (50% group), which is nearly 5% difference. Although not statistically significant, such a difference in feed utilisation may be of significant economic importance for the farmer. Thus, the present data indicates that hypoxic periods are undesirable for optimal feed utilisation.

Table 5 Feed efficiency ratio (FER) and feed conversion ratio (FCR) of Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3).

	80	**70**	**60**	**50**	**P-value**	**R^2**
FER:						
Period 1	1.31±0.06	1.34±0.02	1.33±0.02	1.31±0.08	0.9992	0.0000
Period 2	1.16±0.05	1.17±0.01	1.11±0.06	1.04±0.09	0.1317	0.2123
Total trial	1.24±0.03	1.25±0.02	1.22±0.02	1.19±0.03	0.0984	0.2493
FCR:						
Period 1	0.77±0.04	0.75±0.01	0.75±0.01	0.77±0.05	0.9605	0.0003
Period 2	0.87±0.03	0.85±0.01	0.91±0.05	0.98±0.09	0.1187	0.2256
Total trial	0.81±0.02	0.80±0.01	0.82±0.01	0.84±0.02	0.0999	0.2474

3.3 Apparent digestibility (ADC)

The ADC of nitrogen, lipids and energy in the control group was comparable to that found in other studies with Atlantic salmon (Carter & Hauler, 2000;Aas *et al.*, 2006;Kraugerud *et al.*, 2007). The ADC of ash was variable with negative values. This is as expected for fish kept in sea water, since the fish drink water which adds minerals to the digesta.

There were no significant treatment effects on the ADC of any of the analysed nutrients. This is in accordance with Pouliot and De la Noue (1989) who found no effect of eight-days hypoxic periods on digestibility of dry matter, protein and energy in rainbow trout. In the present trial thus, there were no indications that the fluctuating oxygen saturation affected the digestibility of macronutrients when both daily feedings occurred during normoxia. It should be noted that faeces was collected only at final sampling, and thus, the digestibility estimation is based on this point in time only. Possible adaption or changes in capacity of digestibility during the course of the trial is not picked up in this measurement.

Table 6 Apparent digestibility (ADC, %) of nutrients in the experimental diet fed to Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3).

	80	70	60	50	P-value	R^2
Dry matter	74.7±2.0	76.4±0.7	75.7±0.8	74.5±0.9	0.6945	0.0161
Nitrogen	89.9±0.2	90.5±0.3	90.6±0.3	90.2±0.4	0.3104	0.1025
Lipids	96.4±2.4	97.1±0.4	97.2±0.3	96.7±0.6	0.7201	0.0134
Ash	-19.4±11.1	-8.4±6.5	-14.5±9.9	-22.0±6.8	0.5821	0.0313
Energy	87.9±1.0	88.8±0.2	88.7±0.4	88.3±0.6	0.5157	0.0434

3.4 Body composition and retention

At termination of the trial, there was no significant treatment effect on the whole body content of dry matter, nitrogen and ash. The lipid content however, was significantly negatively affected by reduced oxygen saturation during hypoxia. Corresponding results were found for retention of these nutrients. The deposition of lipid corresponds to feed intake, indicating that fish with the highest feed intake deposited most lipid.

Table 7 *Whole body composition (% or MJ/kg) from start of the trial, and of Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3). At start, 3 fish per tank were sampled and pooled. At termination of the trial, 10 fish per tank were sampled and pooled by tank. The data from start of the trial was not included in statistical analyses.*

	Start	80	70	60	50	P-value	R^2
Dry matter	29.73	32.41±0.44	33.04±0.21	32.50±0.51	32.00±0.85	0.2821	0.1144
Nitrogen	2.92	3.01±0.07	2.94±0.09	2.93±0.05	3.01±0.01	0.9502	0.0004
Lipids	9.6	12.5±0.4	13.0±0.3	12.3±0.2	11.7±0.7	0.0429	0.3496
Ash	2.27	2.06±0.05	2.02±0.13	2.11±0.05	2.09±0.14	0.5225	0.0421
Energy	8.11	9.17±0.12	9.41±0.03	9.22±0.06	8.95±0.28	0.1353	0.2088

Table 8 *Retention (%) of ingested and digested dry matter, nitrogen, lipid and energy in Atlantic salmon kept at stable oxygen saturation (80%, control), or exposed to four daily drops in oxygen level to 70, 60 or 50% oxygen saturation (mean ± S.E.M., n=3). "Lipid retention" includes lipids synthesized from non-lipid precursors.*

	80	70	60	50	P-value	R^2
Retention of ingested material:						
Dry matter	43.9±0.7	45.5±0.5	44.3±0.6	42.8±1.2	0.2695	0.1202
Nitrogen	52.8±2.5	50.4±2.2	49.9±1.4	52.2±0.6	0.7799	0.0082
Lipids	60.9±0.3	64.6±0.8	59.9±0.9	55.6±3.3	0.0551	0.3202
Energy	49.6±0.8	51.9±0.8	50.3±0.6	47.7±1.7	0.2150	0.1491
Retention of digested material						
Dry matter	58.9±1.7	59.5±0.8	58.5±0.4	57.5±1.6	0.3633	0.0832
Nitrogen	58.7±2.8	55.7±2.4	55.1±1.7	57.9±0.5	0.7301	0.0124
Lipids	63.2±0.8	66.5±0.7	61.6±0.9	57.5±3.2	0.0383	0.3625
Energy	56.5±1.2	58.4±1.0	56.8±0.8	54.0±1.8	0.1589	0.1882

3.5 Conclusion

Based on the present dataset, the salmon seemed to tolerate the hypoxic periods in the trial. However, feed intake was reduced as oxygen saturation during hypoxic periods was reduced, and thus growth was reduced correspondingly. The deposition of fat was also reduced correspondingly. The apparent digestibility of macronutrients was not affected by the hypoxic periods. It should be noted that the growth was suboptimal, and the effects of hypoxia may be different when the growth rate is higher than in this trial.

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