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Production performance of Atlantic salmon post-smolts in cyclic hypoxia, and following compensatory growth

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Running title: Feeding and growth of Atlantic salmon in cyclic hypoxia

Abstract

The present study investigated production performance of post-smolt Atlantic salmon (*Salmo salar* L.) subjected to cyclic oxygen reductions (hypoxia) of varying severity. Triplicate groups (N=955), were kept at constant 80% O_2 (control) or subjected to 1 h and 45 minutes of hypoxia (50, 60 or 70% O_2, termed 80:70, 80:60 and 80:50 groups) every 6 h at 16 °C for 69 days. Feed was provided in normoxia. One third of the fish were kept further for 30 days in normoxia to study possible compensatory growth. Cyclic hypoxia did not alter the oxygen uptake rates of fish, measured in nighttime. Fish subjected to 50 and 60% O_2 reduced feeding by 13 and 6% compared to the controls, respectively, with corresponding reductions in specific growth rates. Feed utilization was not reduced. Compensatory growth was observed in fish from the 80:50 group, but full compensation was not achieved. The main conclusions were that feeding in normoxia does not fully alleviate negative effects of cyclic hypoxia on feeding and growth, when oxygen is reduced to 60% or below in hypoxic periods, that feed utilization is maintained, and that compensatory growth may lessen negative effects.

Introduction

Oxygen is the main limiting factor of fish metabolism (Fry 1971), and adequate oxygen supply is therefore essential for optimal welfare and growth performance in Atlantic salmon (*Salmo salar* L.) aquaculture. Any water oxygen saturation that reduces the aerobic metabolic scope of fish is defined as environmental hypoxia (Farrell & Richards 2009). In late summer and autumn, low levels of oxygen (30-70% O_2) has been found to occur in sea cages in the coastal areas of Western Norway, in cycles that resembles the turns of the tidal water current (Johansson, Ruohonen, Kiessling, Oppedal, Stiansen, Kelly & Juell 2006; Johansson, Juell, Oppedal, Stiansen & Ruohonen 2007; Oppedal, Dempster & Stien 2011). The observed oxygen levels are below the suggested oxygen minima for maintained growth of salmonids (70-100% at 16 °C) (Davis 1975; Wedemeyer 1996; EFSA 2008), and may therefore reduce production performance and impair fish welfare. However, a recent study on Atlantic salmon subjected to cyclic hypoxia, showed that acclimated fish utilized normoxic periods for feeding (Remen, Oppedal, Torgersen, Imsland & Olsen 2012), suggesting that negative effects on growth can be minimized by providing feed in normoxia. In order to establish safe limits for oxygen, it is necessary to understand how the production performance of Atlantic salmon is affected by cyclic hypoxic periods, when feed is provided in normoxic periods.

Reduced feed intake is a well-known response of salmonids subjected to hypoxia (e.g. Brett 1979; Bernier & Craig 2005; Glencross 2009; Remen *et al.* 2012), and results in growth depression if hypoxia is frequent or prolonged (e.g. Brett 1979; Crampton, Hølland, Bergheim, Gausen & Næss 2003; Glencross 2009). When hypoxia occur in short-term periods, it has been shown in both Atlantic salmon (Remen *et al.* 2012) and turbot (*Scophthalmus maximus* L.) (Person Le-Ruyet, Lacut, Bayon, Le Roux, Pichavant & Quemener 2003) that appetite varies with the experienced oxygen saturation. To what extent appetite is regained in normoxic periods, can be expected to depend on the severity and duration of hypoxic periods. For example, when fed fish enter hypoxia, digestive processes may be slowed down according to the depression of post-prandial metabolism, and compensated for by an extension of the post-prandial period (Jordan & Steffensen 2007). This response may in turn reduce appetite accordingly due to the prolonged presence of feed in the intestine (see review by Wang, Lefevre, Huong, Van Cong & Bayley 2009). In severe hypoxia, both a general stress response (Bernier & Craig 2005; Remen *et al.* 2012) and recovery from anaerobiosis (Lewis, Costa, Val, Almeida-Val, Gamperl & Driedzic 2007) may result in lowered appetite after return to normoxia.

If growth is reduced as a result of cyclic hypoxia, this effect may be alleviated by an acceleration of growth when hypoxic periods come to an end (see review by Ali, Nicieza & Wootton 2003). Such compensatory growth has been observed in turbot and spotted wolffish (*Anarhichas minor* O.) after being subjected to long-term, continuous hypoxia (Person Le-Ruyet *et al.* 2003; Foss & Imsland 2002), but has not been studied in Atlantic salmon.

The aim of the present study was to investigate the effect of cyclic hypoxia severity on feed intake, feed utilization, metabolism and growth of Atlantic salmon. Water temperature (16 ˚C) and the duration (1 h 45 min) and frequency (every 6 h) of hypoxia were set to mimic oxygen drops that may occur in sea cages during the turn of tidal currents in late summer and autumn. Growth was followed for 30 days after cessation of cyclic hypoxia, in order to study possible compensatory growth.

Materials and Methods

Fish material and experimental facilities

The experiment was carried out at the Institute of Marine Research, Matre, Norway using Atlantic salmon post-smolts (*Salmo salar* L., AquaGen strain) hatched in January 2008. Out-of-season smolts were produced according to standard procedures. This involves constant illumination (LL) from first-feeding until smoltification was initiated by a winter signal (6 weeks of L:D, 12:12). The parr-smolt process was completed by another 6 weeks of LL before sea transfer on September 22nd 2008 (e.g. Oppedal, Juell & Johansson 2007). On February 9-10th 2009, approximately 1300 post-smolts (209±1 g; mean±SEM) were tagged with individual Floy® tags and distributed among 12 indoor circular tanks (Ø=3 m, ~5600L) supplied with 9 ºC sea water (34 g L^{-1}). The temperature was gradually increased to 16 ºC by March 28th and kept constant throughout the cyclic hypoxia period. Illumination was constant and provided by one fluorescent light tube (Philips, TL-D 36W/33-640) per tank. Feed was distributed by Arvotec feeding units (Arvo-Tec T drum 2000, www.arvotec.fi). Feeding, tank water flow and temperature were automatically controlled from custom made computer software (SD Matre, Normatic AS, Nordfjordeid, Norway). Oxygen (Oxyguard 420 probe, Oxyguard International, Denmark, http://www.oxyguard.dk), temperature (TST 487-1A2B temperature probes), salinity (Liquisys MCLM223/ 253 probes) and flow (Promag W flow meters, Endress + Hausser) were measured continuously and a mean for every 5 minutes recorded at tank level. Oxygen probes were re-calibrated in air every 7 days. Prior to experimental start-up, a minimum of 80% O_2 was maintained in tank outlets. Oxygen levels were controlled by managing water inflow rates at all times during the experiment.

Experimental design

The experiment was divided into two separate periods; the cyclic hypoxia-period (days 1-69) and the post-hypoxia period (days 70-99).

The cyclic hypoxia period was initiated on April 24th 2009 (day 1) using four triplicate tank groups of individually tagged Atlantic salmon post-smolts (overall initial weight 383±2 g; mean±SEM, see Table 3). The control group was kept at constant 80% O_2 saturation (referred to as 80:80 and "normoxia"). The treatment groups were subjected to cyclic oxygen reductions (lasting 1 h 45 min, every 6 h), from 80% O_2 saturation, to either 50% (80:50), 60% (80:60) or 70% O_2 saturation (80:70; all levels referred to as "hypoxia") (see Table 1, Fig. 1). During the hypoxic periods, the water current was maintained using a submerged pump (capacity of 120 L min^{-1}) varying in supply depending on the amount of inflowing water. The transition periods between normoxia and hypoxia lasted for approximately 1 h 10 minutes. Hypoxic periods started at 04:30, 10:30, 16:30 and 22:30 daily.

The post-hypoxia period (compensation) was initiated on day 70 using one third of the post-smolts (overall initial weight 791±9 g, see Table 5) randomly taken from two of the replicated tanks. To avoid extension of possible tank effects, individually tagged fish were redistributed into 6 experimental tanks, mixing fish from all groups within each tank in a common garden design. Oxygen was maintained at ~ 90% O_2 and temperature at ~17 °C (see Table 1).

Feed and feeding

Prior to the experiment, salmon were fed commercial feed (Skretting Nutra 2 and 3, and BioMar CPK 75 and 200). On March 26th, experimental feed, produced at Nofima (Bergen, Norway) was introduced. The feed (4.5 mm) was based on high quality fish meal and fish oil (see Table 2). 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, Storebakken, Thomassen, Refstie & Thomassen 2000). Feeding lasted 20 minutes twice daily during normoxia (starting at 08:20 and 14:20, see Figure 1) with a dose aiming at 20% overfeeding. Following every meal, uneaten pellets were collected and feed intake estimated as described by Helland, Grisdale-Helland & Nerland (1996). During the post-hypoxia period, an overfeeding of 20% was maintained, but feed spill was not recorded. This was not done because fish from different groups were mixed in each tank, making it impossible to calculate the feed intake in experimental groups.

Samplings

Weights and lengths of individually tagged fish were recorded at start (days -1 to 0), on days 34-35, at end of the cyclic hypoxia period (days 69-70) and at end of the post-hypoxia period (day 99). Fish were fasted 24 h prior to sampling. To reduce handling stress, a pre-sedation regime was used in the holding tanks. Water level was reduced to 1/3 and Finquel®

(Scanaqua, Årnes, Norway, 20 mg L^{-1}) added. Fish were then calmly netted into a full strength anesthetic bath (Finquel, 60 mg L^{-1}) with oxygen supply prior to identification, weight and length recordings to the nearest g and 0.5 cm length. Nine fish per group (3 fish per tank, pooled as three replicates) at start, and 10 fish from each tank (pooled by tank) at termination of the trial were sampled for analyses of the whole body content of nutrients.

During sampling on day 34-35, 6-9 fish were randomly removed from all tanks to reduce biomass and to maintain the water flow required for 80% O_2 saturation in tanks during normoxia.

One week prior to the end of the cyclic hypoxia period (day 62), 30 fish per tank were stripped for faeces (samples pooled by tank) as described by Austreng (1978). To reduce the risk of empty intestines at faecal sampling, the salmon were fed every six hours during the last day and night prior to sampling (08:10, 14:10, 20:10 and 02:10 for tank 1). Feeding and hypoxic periods for each tank were re-set into a staggered manner (15 or 20 minutes delay from one tank to another), to ensure that the time period between feeding and sampling did not exceed 6.5 h. Fish were pre-sedated and sampled as described above and returned after stripping. Faeces were stored at -20 ºC and freeze dried prior to chemical analysis. During faeces sampling (day 62), some maturing fish were observed. Therefore, gonad weights and sex were noted at samples thereafter (see Table 2 and Table 3), in order to investigate the correlation between GSI and growth of fish.

Chemical analyses

Feed, faeces (freeze dried) and whole body were analysed for crude lipids (Soxtec HT6, Tecator, Höganäs, Sweden), nitrogen (Kjeltec Auto System, Tecator, Höganäs, Sweden), ash (550 ºC until constant weight), dry matter (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).

Recordings of MO_2 during normoxic and hypoxic periods

Oxygen consumption rates (MO_2, mg kg^{-1} min^{-1}) were recorded at 5 min intervals in each tank throughout the cyclic hypoxia period, using the following formula:

$$MO_2 = ((V \times (O_{2\,t\text{-}5} - O_{2\,t})\ 5\ \text{min}^{-1}) + (F \times (O_{2\,\text{in}} - O_{2\,t}) + O_{2\,\text{flux}}) \times BM^{-1},$$

where $O_{2\,in}$ is the oxygen concentration (mg L^{-1}) in the tank inlet, $O_{2\,t}$ is the oxygen concentration (mg L^{-1}) measured in the tank outlet at time *t* (min), *V* is tank water volume (L), *F* is water inflow rate (L min^{-1}), $O_{2\,flux}$ is the influx of oxygen (mg min^{-1}) over the tank water surface and BM is the biomass in the tank (kg; see *Calculations*). The influx was found empirically by measuring oxygen influx in the experimental tanks with oxygen-stripped (N_2 gas was used) water, and with no fish. The resulting formula was $O_{2\,\text{flux}} = k \times (100\% - O_{2\,t\,\%}) \times S \times V \times 100^{-1})$, where *k* is the diffusion constant, $O_{2\,t\,\%}$ is the oxygen saturation measured in the outlet at time *t*, *S* is the solubility of oxygen (mg L^{-1}) and *V* is volume (L). The diffusion constant was determined to be 0.00135, by finding the value of k that maximized the correlation between the observed and modeled increase in oxygen saturation after oxygen-stripping (R^2=0.9997).

To calculate MO_2 during normoxia and hypoxia in the different groups, and to estimate the difference in MO_2 between normoxia and hypoxia, the mean MO_2 was calculated for 1 h during normoxia (01:30-02:30) and 1 h during hypoxia (05:00-06:00) on a daily basis. These periods were chosen because disturbances such as feeding, cleaning and calibration of oxygen probes were minimal during these periods.

Calculations

Feed conversion ratio (FCR) was calculated using

$$\text{FCR} = \text{Feed eaten} \times \text{weight gain}^{-1}.$$

Specific growth rate (SGR) was calculated according to

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

where $g = (\ln W_2 - \ln W_1)\ (t_2 - t_1)^{-1}$, and where W_1 is the weight at the start of the growth period (t_1) and W_2 is the weight at end (t_2) (Houde & Schekter 1981).

Condition factor (CF) was calculated by the formula

$$\text{CF} = 100\text{WL}^{-3},$$

where W is the weight (g) and L is the fork length (cm) of the fish.

Apparent digestibility (ADC, %) was calculated as

$$\text{ADC} = 100(a - b) \times a^{-1},$$

where a is the nutrient to marker (Y_2O_3) ratio in diet and b is the nutrient to marker ratio in faeces.

Nutrient retention (R, % of digested) was calculated using the formula

$$R = 100(\text{Nutrient content at end - Nutrient content at start}) \times \text{Nutrient digested}^{-1},$$

with all measurements in grams. “Lipid retention” includes whole-body lipid from non-lipid precursors.

Tank biomass on days between samplings (BM, kg) was estimated using

$$\text{BM} = \text{BM}_{\text{day-1}} + \text{FI}_{\text{day-1}} \times \text{FCR}^{-1},$$

where BM_{day-1} is the biomass on the previous day, FI_{day-1} is the total daily feed intake (g DM) during the previous day and FCR is the feed conversion ratio in the period between samplings.

Statistics

Statistical analyses were performed using Statistica© (StatSoft, Inc., USA). Effects of treatments on repeated measurements of individual weights, lengths, condition factors and specific growth rates were analyzed using MANOVA (Johnson & Wichern 1992). Significant MANOVAs were followed by a three-way nested ANOVA (Zar 1996), in order to investigate the effects of treatments, replicates (tanks; nested in treatment) and sex in each growth period. Non-significant factors were sequentially removed from the analysis. Effects of treatments on total daily feed intake were analyzed using ANCOVA (Zar 1996), with treatment as categorical predictor and day number as continuous predictor. Effects of treatments on feed utilization parameters (FCR, apparent digestibility, retention and whole body composition of energy and nutrients) were analyzed using One-Way ANOVA. Significant ANOVA/ANCOVAs were followed by Student-Newman-Keuls multiple comparison tests to determine differences between groups. Effects of treatments on MO_2 in hypoxic and normoxic periods were analyzed using regression analysis. The effects of treatments on male and female GSI's were analyzed using Kruskal-Wallis ANOVA rank test. The correlation between gonadosomatic indexes (GSI) and specific growth rates of males and females were analyzed using Spearmans rank order correlation test. A significance level of 5% was used.

Results

Oxygen consumption rates

There were no effects of treatments on oxygen consumption rates (MO_2) in hypoxic periods or normoxic periods (Fig. 2A-B). Similarly, there were no differences in MO_2 between normoxic and hypoxic periods within either treatment groups.

Feed intake, feed utilization and growth during the cyclic hypoxia period

The negative effect of cyclic hypoxia on feed intake was most pronounced for the first period (days 1-34, Fig. 3). In this period, feed intakes of fish from 80:50 and 80:60 groups were reduced by 13% and 10% compared to the controls, respectively. Corresponding, albeit non-significant, reductions in specific growth rates (SGR) were observed (14 and 11% reductions in 80:50 and 80:60 groups, respectively, see Fig. 4). During the second period (days 35-69), fish in the 80:70 group had the highest feed intake (6% higher than in controls), while fish in the 80:60 group ingested the same amount as controls. For fish subjected to 50% O_2 in hypoxic periods, feed intake remained 13% lower than the controls (Fig. 3). Effects on SGR were similar, but non-significant (SGR reduced by 13% in fish from the 80:50 group, see Fig. 4).

Overall, the 68 days of cyclic hypoxia led to significant growth reductions in fish from the 80:50 group (13% lower compared to controls) and the 80:60 group (6% lower compared to controls, see Fig. 4). SGR was highly correlated to feed intake ($R^2=0.83$, $p<0.01$), but not correlated to feed conversion ratio ($R^2=0.23$, $p=0.11$). Although not statistically significant ($p=0.080$), weights of fish in the 80:50 and 80:60 groups were reduced by 10 and 6% compared to the controls, respectively (Table 3). Fish in the 80:70 group were slightly smaller than the control at start (5% lower weight, $p=0.097$), and had a growth rate that was higher than the control during the cyclic hypoxia period (4% increase, see Fig. 4). The negative effects of cyclic hypoxia on fish lengths were borderline significant ($p=0.053$), while no effects on condition factors were observed ($p=0.761$, see Table 3).

Compared to the control, there were no effects of treatments on feed conversion ratios (Fig. 5), apparent digestibility coefficients, retentions and whole body contents (WBC) of nitrogen, lipids and energy (Table 4). There were however significant reductions in the WBC of energy, and the retentions of energy and lipids, in fish from the 80:50 group compared to the fish with highest growth rates; the 80:70 group. WBC of energy was reduced by 8%, whilst retention of energy and lipids were reduced by 14 and 10%, respectively (Table 4).

Weak, but significant positive relationships between gonado-somatic indexes (GSI) and growth rates were observed during days 1-34 (R^2=0.19 and 0.15 for males and females, respectively), and negative relationships were observed during day 35-69 (R^2=0.35 and 0.30 for males and females, respectively). Growth rates of males were lower than in females in the latter period (Fig. 4). There were no effects of treatments on GSIs, with one exception: GSIs were lower in males of the 80:60 group compared to the 80:50 group during days 35-69 (Tables 3 and 5).

Growth during the post-hypoxia period

One third of the fish used in the cyclic hypoxia period were followed in the post-hypoxia period to in order to study possible compensatory growth. Fish from the 80:50 group grew significantly faster than the controls (51% higher SGR) during this period, while fish in 80:60 and 80:70 groups displayed SGR's similar to the controls (Fig. 6). The accelerated growth of fish in the 80:50 group reduced the weight differences compared to the controls from 13 to 9% (Table 5). The overall growth rates for the entire experimental period (days 1-99) did not differ between groups (Fig. 6).

Discussion

Data from the present experiment showed that feeding in normoxia is not sufficient to fully alleviate the negative effects of hypoxic periods on feed intake of salmon post-smolts. Fish were able to compensate for cyclic reductions in oxygen to 70% O_2, but not when oxygen was reduced to 60 and 50% O_2. The more pronounced effect observed in fish subjected to 50% O_2 agree with an increased limitation of metabolism as oxygen declines (Fry 1971). The depression of appetite in fish subjected to 50 and 60% O_2 was lower in the present experiment (13% and 6% reductions, respectively) than in the study by Remen *et al.* (2012), where post-smolts were fed in both hypoxic and normoxic periods (33 and 9% reductions, respectively). Thus, it is considered beneficial to provide feed in normoxic periods if cyclic hypoxia occurs.

The reduced feed intake was not a direct effect of the oxygen level during feeding, as all fish were fed in normoxic periods (80% O_2). Rather, the negative effect on feed intake reflected inadequate oxygen levels in hypoxic periods. To start with, the oxygen level in hypoxic periods may not have been sufficient to support post-prandial metabolism. In Atlantic cod (*Gadus morhua* L.), a depression of post-prandial metabolism was found to prolong the post-prandial period (Jordan & Steffensen 2007). This again may reduce the appetite during following meals due to a prolonged reduction of the metabolic scope and increased presence of food in the intestine (Wang *et al.* 2009). Secondly, if oxygen was reduced below the anaerobic threshold during hypoxic periods, this may have reduced feed intake in normoxic periods due to stress developed during hypoxia (Bernier & Craig 2005; Remen *et al.* 2012) and energy-demanding recovery processes (e.g. lactate removal) upon return to normoxia (Lewis *et al.* 2007).

The anaerobic threshold is thought to lie around P_{crit}, the oxygen threshold where oxygen uptake rates of fish goes from being independent of oxygen availability to decrease with a further reduction in oxygen (Richards 2009). The similar oxygen uptake rates of fish during hypoxic and normoxic periods, suggest that oxygen was not reduced below P_{crit} in nighttime hypoxic periods. This agrees with results of Barnes, King & Carter (2011), who found that P_{crit} of fasted (12 h) Atlantic salmon parr was ~3.4 mg l^{-1} at 14 and 18 °C (corresponding to 43 and 47% O_2, respectively). However, it has been shown that lactate starts to accumulate at 60% O_2 in fed Atlantic salmon post-smolts kept at 16 °C (Remen *et al.* 2012), suggesting that P_{crit} may be considerably higher in fed fish (Richards 2009).

The lack of effect of cyclic hypoxia on apparent digestibility of nutrients and feed conversion ratios is in accordance with results of Glencross (2009) and Pouliot & De La Noüe (1989), who found that nutrient utilization was not impaired in rainbow subjected to continuous hypoxia (40 and 56% O_2). It should however be noted that possible early effects on digestion, and changes in digestive capacity during the experiment, are not picked up by the digestibility estimation performed on day 62. The reduced deposition of lipids and energy in fish from the 80:50 group compared to fish in the 80:70 group, can be related to the difference in feed intake and growth, which was found to be largest between these two groups (see Ali *et al.* 2003, for review).

The negative effects of cyclic hypoxia on growth rates of post-smolts were explained by reduced feed intake, and not reduced feed utilization. This is in accordance with results from studies on juvenile turbot (Pichavant 2001), spotted wolffish (Foss & Imsland 2002) and European sea bass (*Dicentrarchus labrax* L.) (Thetmeyer, Waller, Black, Inselmann & Rosenthal 1999; Pichavant 2001) subjected to hypoxia. The observed effects on growth show that the oxygen minimum for maintained growth lies between 60 and 70% O_2, when hypoxia occur in tidal cycles at 16 °C. Thus, it occurs that salmon post-smolts tolerate repeated, short-term reductions in oxygen below suggested oxygen minimums (70-100% at 16 °C) (Davis 1975; Wedemeyer 1996; EFSA 2008), as long as oxygen is not reduced to levels around or below the anaerobic threshold (~60% O_2 in fed fish at 16 °C; Remen *et al.* 2012). It should be noted that sexual maturation may have reduced the magnitude of negative effects on growth in the present experiment, due to increased individual variation and reduced overall growth (see Fjelldal, Hansen & Huang 2011, for the stimulating effect of continuous light and elevated temperature on sexual maturation). However, a critical limit for growth between 60 and 70% O_2 is considered trustworthy, due to the close accordance with results from a similar experiment by Remen *et al*. (2012). In their experiment, a reduction in oxygen from 70 to 60% O_2 entailed an emerging accumulation of lactate, and a change in feeding pattern towards depressed feed intake in hypoxia and compensatory feeding in normoxia.

The magnitude of growth reduction (13%) in fish subjected to 50% O_2 implies that measures should be taken to avoid frequent reductions in oxygen to such levels. To begin with, the suboptimal growth represents a cost to the farmer, in terms of reduced slaughter weights or a prolonged production period in sea cages. But another important reason is that the health (Wendelaar Bonga 1997) and welfare (Farm Animal Welfare Council 1996) of fish is compromised due to the oxygen shortage and stress observed at this oxygen level (Remen *et al.* 2012).

The marked increase in growth rates of fish in the 80:50 group compared to the control during the post-hypoxia period, show that compensatory growth occurred. This is a well-known response of fish returned to favorable conditions after a period of suboptimal environmental conditions and depressed feed intake (see Ali *et al.* 2003 for review). A period of 30 days with normoxic conditions were however not sufficient for fish in the 80:50 group to reach the same weights as fish in the control group. Relying on compensatory growth to alleviate negative effects of cyclic hypoxia on growth is therefore not considered a favorable strategy with regard to production efficiency in salmon farming. The present results are in accordance with accelerated growth observed in turbot and spotted wolffish returned to normoxic conditions after being subjected to long-term, continuous hypoxia (Foss & Imsland 2002; Person Le-Ruyet *et al.* 2003).

Conclusions

The results from the present experiment show that feeding in normoxia does not fully alleviate the negative effects of cyclic hypoxia on production performance. Such a feeding strategy is however considered beneficial compared to feeding in both hypoxic and normoxic periods. Feed utilization was maintained, and growth was reduced according to feed intake. The oxygen threshold for maintained growth, when oxygen reductions occur in tidal cycles, was found to lie between 60 and 70% O_2. Compensatory growth may lessen negative effects after cessation of hypoxic periods.

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Figure legends

Fig. 1. Schematic overview over the daily fluctuations in oxygen saturation (%) in each of the four experimental groups during the cyclic hypoxia period. Shaded areas represent feeding periods and tanks were exposed to continuous lighting. Hypoxic periods were introduced every 6 hours throughout the cyclic hypoxia-period and fish were fed in normoxic periods only.

Fig. 2. A-B. Oxygen consumption rates (MO_2, mg kg^{-1} min^{-1}) of Atlantic salmon post-smolts (*Salmo salar*) in nighttime normoxia (open circles) and following hypoxia (triangles) during days 1-34 (A) and days 35-69 (B) of the cyclic hypoxia period. Regression lines are drawn for normoxic periods (lines) and hypoxic periods (broken lines) and results from regression analyses are presented in the figures (NS=not significant). Values are tank means±SEM (n=34).

Fig. 3. Total daily feed intake (% of biomass) in triplicate tanks of Atlantic salmon post-smolts *Salmo salar* L. subjected to cyclic hypoxia (1 h and 45 min every 6 h) of varying severity (group names indicate percent oxygen saturation in normoxia:hypoxia) at 16 °C. Different lower-case letters denote significant differences between groups within growth periods. Values are group means±SEM (n=3).

Fig. 4. Specific growth rates (SGR, % of body weight per day) of Atlantic salmon post-smolts *Salmo salar* L. subjected to cyclic hypoxia (1 h and 45 min every 6 h) of varying severity (group names indicate percent oxygen saturation in normoxia:hypoxia) at 16 °C. P-values (three-way nested ANOVA) of treatment effect (T), replicates nested in treatment (R(T)) and sex (S) are given for each growth period. Different lowercase letters indicate significant differences between groups. Values are group means±SEM (n=3).

Fig. 5. Feed conversion ratio (FCR) in triplicate tanks of Atlantic salmon post-smolts *Salmo salar* L. subjected to cyclic hypoxia (1 h and 45 min every 6 h) of varying severity (group names indicate percent oxygen saturation in normoxia:hypoxia) at 16 °C, during first 35 days , subsequent 34 days , and overall (days 1-69).

Fig. 6. Specific growth rates (SGR, % of body weight per day) of Atlantic salmon post-smolts *Salmo salar* L. in the post-hypoxia period (day 70-99, 90% O_2), which followed after 68 days of cyclic hypoxia of varying severity (group names indicate percent oxygen saturation in normoxia:hypoxia during the cyclic hypoxia period), and the overall SGR during the entire experimental period (day 1-99). P-values (three-way nested ANOVA) of treatment effects (T), replicates nested in treatment (R(T)) and sex (S) are given for each growth period. Different lower-case letters indicate significant differences between groups. Values are group means±SEM (n=2).

Table 1

Temperature (°C), salinity (g L^{-1}) and oxygen saturation (% of air saturation) in hypoxic periods (O_2 hypoxia) and normoxic periods (O_2 Normoxia) in the four different treatment groups (80:80, 80:70: 80:60 and 80:50) during the cyclic hypoxia period (days 1-69) and overall values during the post-hypoxia period (days 70-99). Values are given as means±SEM (n=3 for days 1-69 and n=6 for days 70-99).

	O_2 Hypoxia (% of air saturation)	**O_2 Normoxia (% of air saturation)**	**Temperature (°C)**	**Salinity (g L^{-1})**
80:80	79.8±0.3	79.7±0.2	15.8±0.0	34.8
80:70	69.6±0.1	79.5±0.1	15.8±0.1	34.8
80:60	59.9±0.3	79.4±0.2	15.8±0.1	34.8
80:50	49.6±0.2	78.2±0.4	15.6±0.1	34.8
Post hypoxia period		89.9±0.1	16.8±0.0	34.8

Table 2

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.

Table 3

Weights (g), lengths (cm), condition factors, total number(N) and gonado-somatic indexes (GSI, % of body weight) in triplicate groups of Atlantic salmon post-smolts at start (days-1- 0) and end (days 69-70) of the cyclic hypoxia period (group names indicate percent oxygen saturation in normoxia:hypoxia). Values are given as means±SEM (n=3). P-values from statistical analyses of treatment effects at start/end are presented, and significant differences between groups are indicated by dissimilar superscript lower-case letters.

Group	**Weight (g)**		**Length (cm)**		**Condition factor**		**GSI at end (% of BW)**		**N**
	Start	*End*	*Start*	*End*	*Start*	*End*	♀	♂	
80:80	391±5	808±12	32.5±0.2	40.2±0.2	1.12±0.02	1.23±0.00	0.22±0.01	2.1±0.1^{b}	225
80:70	371±4	786±13	32.0±0.2	39.5±0.2	1.12±0.03	1.26±0.01	0.19±0.01	1.9±0.3^{ab}	231
80:60	389±6	766±32	32.4±0.0	39.4±0.5	1.12±0.02	1.24±0.01	0.18±0.01	1.6±0.2^{a}	251
80:50	390±7	728±27	32.3±0.1	38.9	1.13±0.02	1.23±0.03	0.31±0.10	2.6±0.2^{b}	248
P-value	*0.097*	*0.080*	*0.200*	*0.053*	*0.926*	*0.761*	*0.148*	*0.003*	

Table 4

Apparent digestibility coefficient (%) retention (% of digested) and whole body composition (MJ/kg) of nitrogen, lipids and energy in Atlantic salmon post smolts at start, and in triplicate groups after 62 days of cyclic hypoxia of varying severity at 16 °C. Group names indicate percent oxygen saturation in normoxia:hypoxia. "Lipid retention" includes lipids synthesized from non-lipid precursors. Values are given as means±SEM (n=3). P-values from the analysis of treatment effects (One-way ANOVA) are given, and significant differences between groups are indicated by dissimilar superscript lower-case letters.

	Apparent digestibility coefficient (%)			**Retention (% of digested)**			**Whole body content (MJ/kg)**		
	Nitrogen	*Lipids*	*Energy*	*Nitrogen*	*Lipids*	*Energy*	*Nitrogen*	*Lipids*	*Energy*
Start							2.9	9.6	8.1
80:80	89.9±0.2	96±2	88±1	59±3	63±1^{ab}	57±1	3.0±0.1	12.5±0.4^{ab}	9.2±0.1^{ab}
80:70	90.5±0.3	97±0	89±0	56±2	67±1^{a}	58±1	2.9±0.1	13.0±0.3^{a}	9.4±0.0^{a}
80:60	90.6±0.3	97±0	89±0	55±2	62±1^{ab}	57±1	2.9±0.1	12.3±0.2^{ab}	9.2±0.1^{ab}
80:50	90.2±0.4	97±1	88±1	58±1	58±3^{b}	54±2	3.0±0.0	11.7±0.7^{b}	9.0±0.3^{b}
P-value	*0.079*	*0.828*	*0.286*	*0.558*	*0.039*	*0.167*	*0.310*	*0.046*	*0.048*

Table 5

Weights (g), lengths (cm), condition factors and total number (N) of Atlantic salmon post-smolts at start (day 70) and end (day 99) of the post-hypoxia period, and gonado-somatic indexes (GSI, % of body weights) at end. Group names indicate percent oxygen saturation in normoxia:hypoxia during the foregoing cyclic hypoxia period. Values are means±SEM (n=2). Results from statistical analyses testing the effects of treatment are presented.

Group	**Weight (g)**		**Length (cm)**		**Condition factor**		**GSI at end (% of BW)**		**N**
	Start	*End*	*Start*	*End*	*Start*	*End*	♀	♂	
80:80	864±44	975±55	40.8±0.7	42.4±0.9	1.26±0.00	1.26±0.01	0.54±0.11	2.6±0.2	83
80:70	788±1	901±1	39.4±0.2	41.3±0.1	1.27±0.02	1.25±0.01	0.35±0.04	3.1±1.1	76
80:60	756±22	867±31	39.2±0.4	41.2±0.2	1.24±0.01	1.23±0.02	0.41±0.11	2.9±0.4	80
80:50	749±43	892±32	39.4±0.0	41.4±0.2	1.21±0.07	1.24±0.02	0.33±0.03	2.7±0.4	75
P-value	*0.170*	*0.270*	*0.134*	*0.297*	*0.745*	*0.326*	*0.397*	*0.334*	

Figure 1

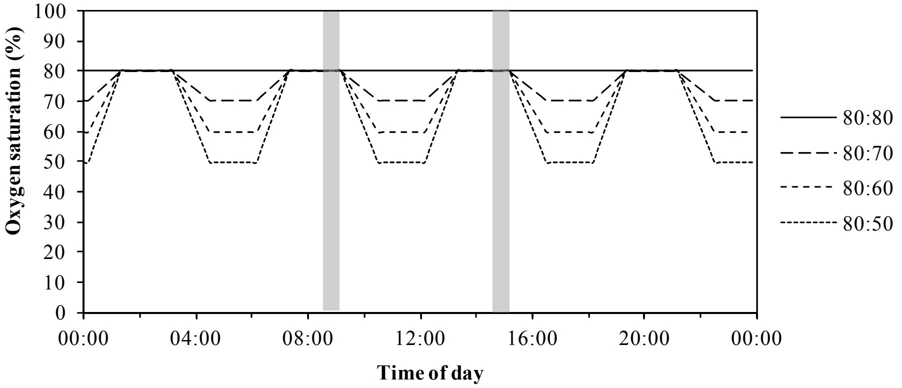

Oxygen saturation (%)
100
90
80
70
60
50
40
30
20
10
0
00:00
04:00
08:00
12:00
16:00
20:00
00:00
Time of day
80:80
80:70
80:60
80:50

Figure 2

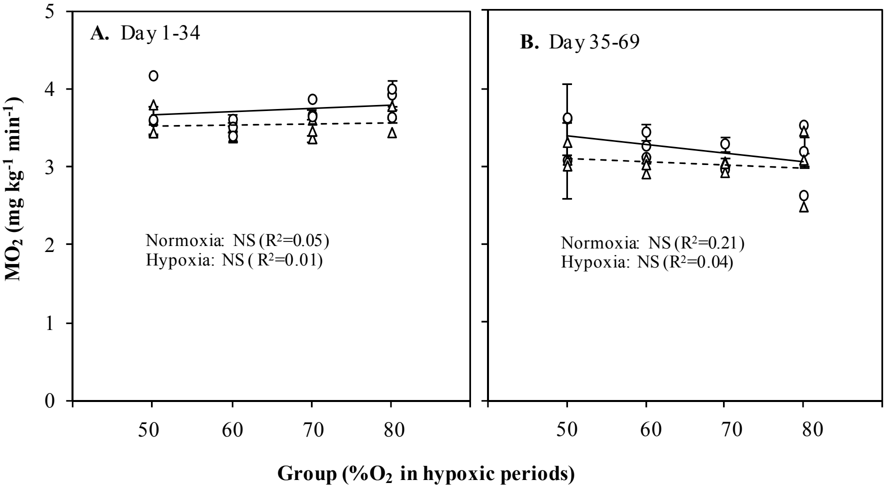

A. Day 1-34
B. Day 35-69
Normoxia: NS (R^2=0.05)
Hypoxia: NS (R^2=0.01)
Normoxia: NS (R^2=0.21)
Hypoxia: NS (R^2=0.04)
MO2 (mg kg-1 min-1)
0
1
2
3
4
5
50
60
70
80
50
60
70
80
Group (%O2 in hypoxic periods)

Figure 3

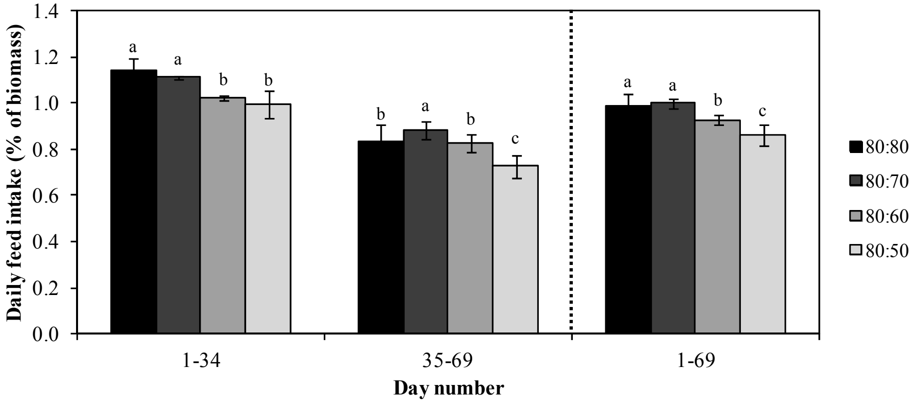

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Daily feed intake (% of biomass)
a
a
b
b
b
a
b
c
a
a
b
c
1-34
35-69
1-69
Day number
80:80
80:70
80:60
80:50

Figure 4

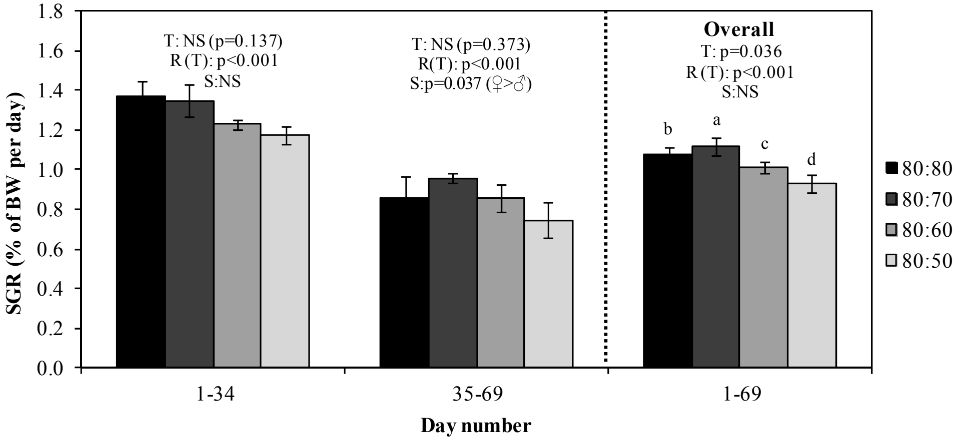

SGR (% of BW per day)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
T: NS (p=0.137)
R (T): p<0.001
S:NS
T: NS (p=0.373)
R(T): p<0.001
S:p=0.037 (♀>♂)
Overall
T: p=0.036
R (T): p<0.001
S:NS
b
a
c
d
■ 80:80
■ 80:70
■ 80:60
□ 80:50
1-34
35-69
1-69
Day number

Figure 5

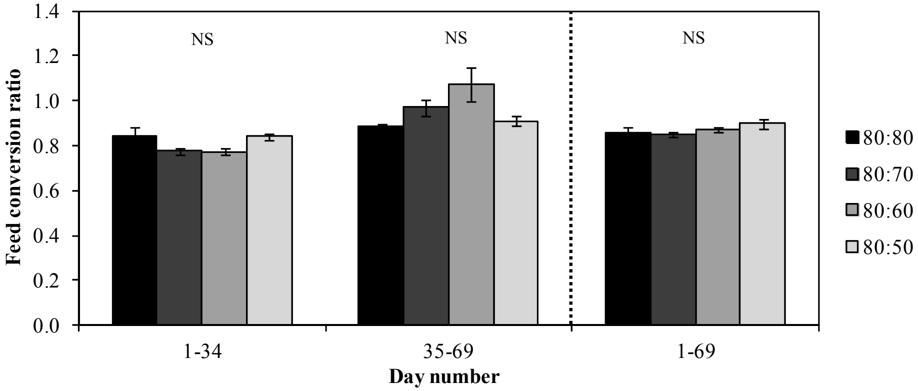
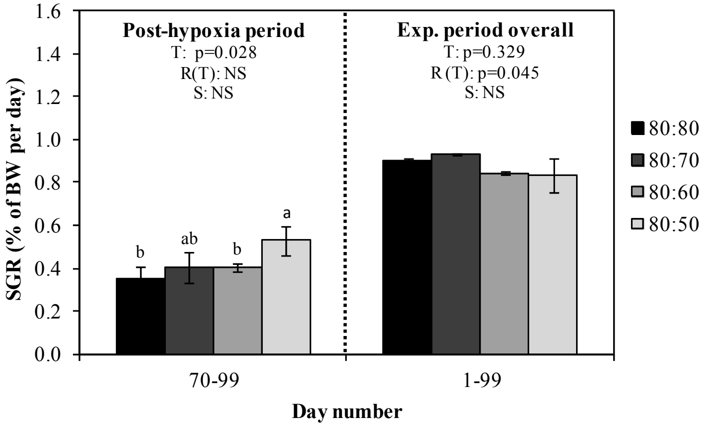

Feed conversion ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NS
NS
NS
1-34
35-69
1-69
Day number
■ 80:80
■ 80:70
■ 80:60
□ 80:50

Figure 6



Post-hypoxia period
T: p=0.028
R(T): NS
S: NS
Exp. period overall
T: p=0.329
R (T): p=0.045
S: NS
b
ab
b
a
80:80
80:70
80:60
80:50
SGR (% of BW per day)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
70-99
1-99
Day number