BORA - UiB
BERGEN OPEN RESEARCH ARCHIVE

This article was made openly available by BORA-UIB, the institutional repository of the University of Bergen. https://bora.uib.no/

This is the author's draft, preprint manuscript of the article:

Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation

Citation published version	Mette Remen, Frode Oppedal, Albert K. Imsland, Rolf Erik Olsen, Thomas Torgersen, Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation, Aquaculture, Volumes 416–417, 5 December 2013, Pages 41-47, ISSN 0044-8486, http://dx.doi.org/10.1016/j.aquaculture.2013.08.024. (http://www.sciencedirect.com/science/article/pii/S0044848613004225)
Link to published version	http://dx.doi.org/10.1109/TIT.2014.2329694
Publisher	Elsevier
Version	Author's preprint/draft version
Citable link	http://hdl.handle.net/1956/9539
Terms of use	Copyright 2013 Elsevier B.V. All rights reserved.
Set statement	NOTICE: this is the author's preprint/draft version of a work. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms are not reflected in this document. Changes have been made to this work since it was submitted for publication. A definitive version was subsequently published in Aquaculture, [416-17, (5.12.2013)] doi:10.1016/j.aquaculture.2013.08.024 ☐

1 **Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency**

2 **of temperature and hypoxia acclimation**

3

4 Mette Remen [1, 2, 3], Frode Oppedal [1, 3], Albert K. Imsland[2, 4], Rolf Erik Olsen[1] and Thomas

5 Torgersen [1]

[1] Institute of Marine Research, NO-5984 Matredal, Norway

[2] Institute of Biology, University of Bergen, Box 7800, N-5020 Bergen, Norway

[3] Centre for research based innovation in aquaculture technology (CREATE), SFI, SINTEF

Sealab, NO-7645 Trondheim, Norway.

6 [4]Akvaplan-niva, Iceland Office, Akralind 4, 201 Kopavogi, Iceland

7

8 Corresponding author: Mette Remen, Tel.:+47 56 36 75 24, e-mail: metter@imr.no

9

12

13

14

15 **Abstract**

16 In order to establish hypoxia tolerance thresholds for Atlantic salmon (*Salmo salar*) in the on-

17 growing phase, the effect of temperature (6, 12 and 18 °C) and hypoxia acclimation (33 days

18 of hypoxic periods occurring every 6 hours at 16 °C) on the oxygen consumption rate (MO_2)

19 and limiting oxygen concentration (LOC; referred to as the hypoxia tolerance threshold) was

20 investigated in fish were kept under production-like conditions (fed, undisturbed and freely

21 swimming fish in tanks). Further, the effects of temperature and oxygen on the relationship

22 between gill ventilation frequency (Vf) and MO_2 were studied in order to evaluate Vf as an

23 indicator of MO_2. Both MO_2 and LOC were found to increase exponentially with temperature

24 (Q_{10} =2.7 for MO_2 and 1.4 for LOC), while hypoxia acclimation resulted in a tendency for

25 reduced MO_2, but no lowering of LOC. The mean LOC at 6, 12, 16 and 18 °C were 2.9, 3.4,

26 3.8 and 4.3 mg L^{-1}, respectively. A strong correlation between MO_2 and LOS (LOC given in

27 units of oxygen saturation) was found (R^2=0.93), regardless of temperature, suggesting that

28 measurements of MO_2 can be used to estimate the LOS of post-smolts. Vf was considered a

29 reliable estimator of MO_2 in normoxic conditions, but not during reductions in oxygen, due to

30 the increasing Vf, and relatively stable MO_2 as oxygen declined towards LOC.

31

1. Introduction

Temperature is the main controlling factor of fish metabolism (Fry, 1947, 1971), and is therefore essential when determining the dissolved oxygen (DO) requirement of cultured fish. The effect of temperature on the DO requirement of Atlantic salmon (*Salmo salar*) in the sea water phase is however largely unknown (reviewed by Thorarensen and Farrell, 2011), and makes it difficult for legislators and aquaculturists to assess whether observed DO levels in sea cages (e.g. Burt et al., 2012; Crampton et al., 2003; Johansson et al., 2006, 2007; Oppedal et al. 2011) are compromising fish performance and welfare.

Rates of biochemical processes and cost of oxygen transport to metabolising tissues increase with temperature (Mark et al., 2002), causing an exponential increase in the standard metabolic rate (SMR, the metabolic rate of fasted and resting fish) with temperature (Brett and Groves, 1979; Farrell et al., 2009). The maximum aerobic metabolic rate (MMR) also increases with temperature at lower and intermediate ranges, but levels off, and eventually decreases, at high temperatures (Farrell et al., 2009; Pörtner, 2010). The metabolic scope for activity, representing the difference between SMR and MMR, therefore increases with temperature up to the point where the increase in MMR no longer keeps up with that of the SMR. This turning point is referred to as the optimum temperature, allowing the largest capacity to feed, digest, assimilate nutrients, swim etc. (Fry, 1947, 1971; Neill and Bryan, 1991). The thermal optimum for Atlantic salmon has been reported in the range of 16 to 20 °C (reviewed by Elliott and Elliott, 2010).

Oxygen is the main limiting factor of fish metabolism (Fry, 1971), and any DO that limit the metabolic scope can be defined as environmental hypoxia (Farrell and Richards, 2009). As DO declines within the hypoxic zone, the oxygen uptake rate can be kept at the same level through increased gill ventilation and perfusion (Barnes et al., 2011; Ott et al., 1980; Perry et al., 2009), but the metabolic scope is gradually reduced as oxygen declines

57   (Fry 1971), causing reduced capacity for feeding and swimming (Kutty and Saunders, 1973;

58   Remen et al., 2012). Eventually, the cost of maintaining MO_2 exceeds the benefit, and MO_2

59   starts to decrease with further reductions in DO (see Perry et al., 2009, for review). Below this

60   threshold, termed the limiting oxygen concentration (LOC) (Neill and Bryan, 1991), the rate

61   of anaerobic metabolism increases sharply, anaerobic end-products accumulate and

62   physiological as well as behavioural stress responses are elicited (Burton and Heath 1980;

63   Perry and Reid, 1994; Remen et al., 2012; Van Raiij et al., 1996; Vianen et al., 2001). Thus,

64   for aquaculture purposes, the LOC for fish with routine MO_2 can be considered the hypoxia

65   tolerance threshold, and constitute a limit for reductions in DO that should be avoided in sea

66   cages due to the hypoxic stress and time-limited survival at such DO levels (Nilsson and

67   Nilsson, 2008).

68   According to the theoretical framework presented by Fry (1971) and reviewed by

69   Wang et al. (2009), the LOC of fish can be expected to increase with any factor that increases

70   the metabolic rate. The LOC of Atlantic salmon in a sea cage can therefore be expected to

71   depend both on water temperature and the metabolic state of the fish (e.g. acclimation state,

72   feeding status, swimming speed and stress level), and determination of LOC for aquaculture

73   purposes therefore requires that the metabolic rate of fish is comparable to that of fish in sea

74   cages. A recent study by Barnes et al. (2011) showed that individual MO_2 was strongly

75   correlated with LOC, regardless of experimental temperature, suggesting that LOC can be

76   estimated from measurements of MO_2. This relationship is useful, as the LOC of Atlantic

77   salmon over a range of temperatures and metabolic states can be estimated, based on MO_2

78   measurements presented in previous studies. However, as the measurements of Barnes et al.

79   (2011) were performed on a relatively small selection of single, fasted fish in a respirometer

80   at high temperatures (14-22 °C), the strong relationship between MO_2 and LOC needs to be

81   validated for a wider temperature range, a larger group of fish and for experimental conditions

82   more similar to the sea cage environment.

83   If LOC is determined by MO_2, it would be of high value to find an easily observable

84   indicator of MO_2 of fish in sea cages, in order to assess whether fish are provided with DO

85   above their LOC, e.g. during short-term reductions in DO (Johansson et al., 2006). Millidine

86   et al. (2008) suggest that gill ventilation frequency (Vf) may serve as an easily observable,

87   and good predictor of MO_2, as a strong correlation between these two variables was found in

88   Atlantic salmon juveniles. However, the effect of declining oxygen on Vf (Perry et al., 2009)

89   was not taken into consideration in the study of Millidine et al. (2008), and the combined

90   effect of temperature and oxygen on ventilation frequency needs to be evaluated in order to

91   discuss the suitability of Vf as an indicator of MO_2.

92   Acclimation to hypoxia has been shown to both reduce the oxygen demand (Pichavant

93   et al., 2000; 2001) and increase the capacity for oxygen uptake and -transport of fish (Lai et

94   al., 2006; Soivio et al., 1980; Tetens and Lykkeboe, 1981). However, in spite of numerous

95   physiological adjustments, LOC was not lowered in Atlantic cod (*Gadus morhua*) after 6-12

96   weeks of acclimation to hypoxia (Peterson and Gamperl, 2010). Correspondingly, a recent

97   study on Atlantic salmon post-smolts suggested that acclimation to periodic hypoxia did not

98   increase hypoxia tolerance considerably, as the depression of feed intake and accumulation of

99   lactate in hypoxia periods was relatively stable for 3 weeks (Remen et al., 2012). It is not

100   known whether LOC is lowered as a result of hypoxia acclimation in Atlantic salmon.

101   The main purpose of this study was to investigate the effect of temperature and

102   hypoxia acclimation on LOC for Atlantic salmon post-smolts kept in experimental conditions

103   resembling production conditions. Further, we aimed to evaluate whether MO_2 determines

104   LOC, allowing LOC estimation based on assessment of MO_2, and whether MO_2 can be

105   estimated from ventilation frequency.

106

2. Material and methods

107

108

2.1. Fish material and experimental conditions

110   This study is based on two separate experiments. The effects of temperature on the

111   oxygen consumption rate (MO_2) and the limiting oxygen concentration (LOC), and the

112   combined effect of temperature and dissolved oxygen concentration (DO) on ventilation

113   frequency (Vf) was studied in Experiment I (referred to as Exp I). The effects of acclimation

114   to hypoxia of varying severity on MO_2 and LOC were studied in Experiment II (referred to as

115   Exp II). Both experiments were carried out at the Institute of Marine Research, Matre,

116   Norway using Atlantic salmon post-smolts (*Salmo salar* L., AquaGen strain) hatched in

117   January 2008. Out-of-season smolts were produced according to standard procedures. This

118   involves constant illumination (LL) from first-feeding until smoltification was initiated by a

119   winter signal (6 weeks of L:D, 12:12). The parr-smolt process was completed by another 6

120   weeks of LL before sea transfer on 22 September 2008 (e.g. Oppedal et al., 2007).

121   In both Exp I and Exp II, the water flow rate, temperatures and feeding (Arvotec

122   feeding units, Arvo-Tec T drum 2000, www.arvotec.fi) in experimental tanks were controlled

123   from custom made computer software (SD Matre, Normatic AS, Nordfjordeid, Norway),

124   which also recorded temperature (TST 487-1A2B temperature probes), flow through rates

125   (Promag W flow meters Endress + Hausser), oxygen level (Oxyguard 420 probe, Oxyguard

126   International, Denmark, http://www.oxyguard.dk) and salinity (Liquisys MCLM223/ 253

127   probes) continuously (1 minute averages). Oxygen probes were calibrated in air once a week.

128   Illumination was constant and provided by one fluorescent light tube per tank.

129

2.2. Experimental design

131   *2.2.1. Experiment I*

132   137 post-smolt Atlantic salmon were transferred from outdoor tanks to indoor, squared

133   tanks (~460 L) fitted with lids on 21 January 2009. Weights (291±4 g) and lengths

134   (28.2±0.1cm) were measured on 11 February (Table 1). Upon transfer, fish were kept in the

135   same water quality as in the outdoor tank (salinity 34 g L^{-1}, temperature 8-9 °C), and

136   temperature was gradually increased (1 °C per day) to 12 °C by 24 January. A water flow

137   through rate of 20 L min^{-1} kept oxygen levels above 7 mg L^{-1} (measured in tank outlet) until

138   30 March, the day before experiment start-up. From this day on and throughout the

139   experiment, oxygen levels were maintained at ~100% of air saturation by an automatically

140   controlled addition of super-saturated sea water (~400% of air saturation), except during LOC

141   measurements.

142   The fish in all four experimental tanks were subjected to three subsequent changes in

143   temperature, from 12 to 18 °C (day 0), 18 to 12 °C (day 20) and 12 to 6 °C (day 29), and were

144   allowed to acclimate to the new temperature for 8-15 days before measurements of MO$_2$ and

145   Vf were performed (days 15, 28 and 42). During the entire experimental period, fish were fed

146   to satiation twice daily (09:30-10:30 and 14:00-15:00), aiming at ~40% surplus of feed. On

147   LOC measurement days, fish were fed to satiation 1-2 h before the initial reduction in oxygen

148   below 100% of air saturation, and the feed intake was estimated according to the method

149   described by Helland et al. (1996). The weights and lengths of fish were recorded on day -48

150   and four days after the last LOC measurement (day 46), following the procedure described in

151   Remen et al. (2012) (see Table 1). Mean weights (±SEM) on LOC measurement days were

152   estimated to be 425±7 g (18 °C), 460±8 g (12 °C) and 501±10 g (6 °C), based on overall

153   specific growth rates.

154

155   *2.2.2. Experiment II*

156   Approximately 1300 post-smolts (209±1 g) were transferred from outdoor tanks and

157   distributed among 12 indoor circular tanks (Ø=3 m, ~5600L) supplied with 9 ºC sea water (34

158   g L^{-1}) on 9-10 February 2009. Temperature was gradually increased until 16 °C was reached

159   on 16 March, and maintained throughout. Flow rates were kept at 80 L min^{-1} and increased to

160   105 L min^{-1} on 26 March, maintaining a minimum of 6.4 mg L^{-1} O_2 (80% of air saturation) in

161   tank outlets prior to the acclimation period.

162   Prior to measurements of MO_2 and LOC, the post-smolts were acclimated to periodic

163   hypoxia of different severities for 33 days at 16 °C. Hypoxic periods were chosen over

164   constant hypoxia, as this is more likely to occur in on-growing production in sea cages (e.g.

165   Burt et al., 2012; Johansson et al. 2006, 2007), and the frequency of hypoxia was set to mimic

166   hypoxic periods occurring during the turn of tidal currents (Johansson et al., 2006). Starting

167   on 24 April 2009, four triplicate groups (tanks) of post-smolts (overall initial weight 383±2 g)

168   were either kept at constant 6.4 mg O_2 L^{-1} (80% of air saturation, referred to as "control" and

169   "normoxia"), or subjected to 1 h and 45 minutes periods of reduced DO every 6 h, to either

170   5.6 (70% of air saturation), 4.8 (60% of air saturation) or 4.0 mg O_2 L^{-1} (50% of air

171   saturation) (Fig. 1). Groups were termed 80:80, 80:70, 80:60 and 80:50, based on the oxygen

172   saturation in normoxia: hypoxia. The desired oxygen levels were maintained by controlling

173   tank water flow rates, while the water current in the tank was upheld using a submerged pump

174   (capacity of 120 L min^{-1}) varying in supply depending on the amount of inflowing water.

175   Throughout the acclimation period, fish were fed to satiation (~25% surplus of feed) twice

176   daily in normoxic periods. Before the LOC measurement on day 33, the latest hypoxic period

177   and the morning feeding period were finished approximately 6 and 4 hours prior to the initial

178   reduction in DO below 100% of air saturation, respectively. The weights and lengths of fish

179   were registered on the day following LOC measurements (Table 1), according to the

180   procedures described in Remen et al. (2012).

181

2.3. Open respirometry

Both in Exp I and Exp II, the oxygen consumption rates (MO_2) of post-smolts were measured during a progressive decline in DO, by using the experimental tanks as open respirometers, in order to find the limiting oxygen concentration (LOC). In brief, the water flow through rates in the holding tanks were reduced to a minimum (some flow was necessary for proper function of oxygen probes), and without disturbing the fish, oxygen gradually declined as a result of fish consumption. This was allowed to continue until the rate of oxygen decline was clearly lowered, indicating that MO_2 was reduced and that LOC had been passed. No fish lost equilibrium during the LOC trials.

In Exp I, DO in tanks was elevated to 115-125% of air saturation by increasing the supply of oxygen-supersaturated water, before the supply was turned off, and the water exchange rate (*Flow*) was reduced to 2 L min^{-1} (12 and 18 °C), or 1 L min^{-1} (6 °C). The oxygen consumption rate per tank (MO_2, mg O_2 min^{-1}) was found from the equation:

$$MO_{2_t} = Vol \cdot Sol \cdot \frac{Sat_t - Sat_{t-1}}{\delta t \cdot 100} + Flow \cdot Sol \cdot \frac{100 - Sat_t}{100},$$

where *Vol* is the tank volume (~460 L) and *Sol* is the solubility of oxygen at prevailing temperature and conductivity conditions. Sat_t is the oxygen saturation at time *t*. The average saturation in 5 minute intervals was used (δt=5 minutes). The oxygen flux over the water surface during the progressive decline in oxygen was investigated by measuring the DO change in tanks without fish, after the water had been oxygen-stripped using N_2 gas. Flow rates corresponded to that used in experiments. The contribution of oxygen flux to the DO development was modeled as

205 $$O_{2_{flux}} = k \cdot (100 - Sat_t) \cdot Sol \cdot \frac{Vol}{100}$$

206   For the lidded experimental tanks in Exp I, the influx was found to be so small that it

207 could not be identified and therefore is considered negligible.

208   In Exp II, DO in tanks was elevated to 110-120% of air saturation by addition of

209 supersaturated water, before this supply was turned off and flow reduced to 3 L min^{-1}. For the

210 large experimental tanks used in Exp II, the influx of oxygen at DO below air saturation was

211 significant, and added to the calculation of tank MO_2. The diffusion constant, k, was estimated

212 to be 0.00135, by finding the value of k that maximized the correlation between the observed

213 and modeled increase in oxygen saturation after oxygen-stripping (R^2=0.9997).

214

215 *2.4. Gill ventilation frequency*

216   The ventilation frequency (Vf, gill movements per minute) was monitored for all LOC

217 measurements in Exp I. Vf was registered in each tank for approximately every 10% decrease

218 in oxygen saturation, by measuring the time needed to perform 14 gill movements in 10 fish

219 and calculating the average.

220

221 *2.5. Calculations and statistics*

222   The specific growth rates (SGR) that was used to estimated weights of fish on LOC

223 measurement days in Exp I, was calculated according to SGR= (e^g-1)100, where $g = (\ln M_2 -$

224 $\ln M_1) (T_2 - T_1)^{-1}$, and where M_1 is the mass at the start of the growth period (T_1) and M_2 is the

225 mass at end (T_2) (Houde and Schekter, 1981). Condition factor (CF) was calculated by the

226 formula CF = $100ML^{-3}$, where M is the mass (g) and L is the fork length (cm) of the fish.

227   The temperature effect of metabolism, Q_{10}, was calculated as

228 $Q_{10} = \left(\frac{MO_{2_{T_2}}}{MO_{2_{T_1}}}\right)^{\frac{10}{T_2 - T_1}}$, where $MO_{2_{T_1}}$ and $MO_{2_{T_2}}$ are oxygen consumption rates (mg kg^{-1} min^{-1})

229 at temperatures T_1 and T_2, respectively (Schmidt-Nielsen, 1997).

230   The break-point in the relationship between ambient DO (mg L^{-1}) and MO$_2$,

231 representing the limiting oxygen concentration (LOC), was found using the "segmented"-

232 package in the free software programme R 2.14.0 (The R Foundation for Statistical

233 Computing © 2011, www.r-project.org). This method simultaneously estimates slope

234 parameters and turning point(s) within a standard linear model framework (Muggeo, 2003;

235 2008) (see Fig. 2A). Maximum number of iterations was set to 30. Only MO$_2$ values for DO $\leq$

236 the concentration equivalent to 90% of air saturation was used at all temperatures, and the

237 normoxic MO$_2$ was determined by averaging all 5 minute values for MO$_2$ above the LOC. A

238 Davies test was used to test for difference in slopes, and results were not included in the

239 manuscript for p>0.05 (Muggeo, 2008).

240   In order to find the ventilation frequency in normoxia (Vf$_{norm}$, at DO equivalent to

241 90% of air saturation at all temperatures), the maximal Vf (Vf$_{max}$) and the limiting oxygen

242 concentration for increased Vf (LOC$_{Vf}$), a third order polynomial relationship was fitted to

243 plots of Vf against DO for each tank using Microsoft ® Office Excel ® 2007 (© 2006

244 Microsoft Corporation). By replacing x in the resulting polynomial function with the DO (mg

245 L^{-1}) equivalent to 90% of air saturation, Vf$_{norm}$ was found. By derivation of the third-order

246 polynomial function and solving the equation for Vf=0, LOC$_{Vf}$ was found. Then, Vf$_{max}$ was

247 calculated by replacing x in the third-order polynomial function with LOC$_{Vf}$ (see Fig. 2B).

248   All further statistical tests were performed using Statistica© (StatSoft, Inc., USA).

249 Fixed non-linear regression was used to test the non-linear relationships between temperature

250 and the parameters feed intake, MO$_2$ and LOC, and the effect of periodic hypoxia severity on

251 feed intake, MO$_2$ and LOC was tested using regression analysis. Differences between LOC

252 and LOC_{Vf} at 6, 12 and 18 °C were tested using One-Way ANOVA. The correlation between

253 MO_2 and LOC was tested using correlation analysis.

254   For comparison of linear relationships between MO_2 and LOC obtained in the present

255 experiment and the study of Barnes et al. (2011), LOC from both studies was expressed as

256 LOS (limiting oxygen saturation, % of air saturation), due to the use of different temperatures.

257 For a given oxygen concentration, the oxygen saturation increases with temperature, and as

258 the saturation (or the corresponding oxygen tension) determines the gradient for oxygen

259 diffusion over the gills (Davis, 1975), this denomination was considered more appropriate

260 than the oxygen concentration for the relatively wide range of temperatures used. It should be

261 noted that one observation from the study of Barnes et al. (2011) was left out of the

262 comparison due to the lower weight (49 g) and long time used to perform the LOC

263 measurement (29 h). Whether the relationship between MO_2 and LOS in the present study

264 differed from that of Barnes et al. (2011), was analyzed using Analysis of Covariance, with

265 study origin as a categorical, random predictor variable, MO_2 as the continuous predictor

266 variable and LOS as the dependent variable.

267

268 **3. Results**

269

270 *3.1. The effect of temperature on feed intake, MO₂ and LOC*

271   The feed intake (FI, % of biomass) of post-smolts during the meal preceding LOC

272 measurements in Exp I increased with temperature, and a logarithmic relationship between

273 temperature and FI was found ($R^2 = 0.93$, p<0.001) (Fig. 3A). The normoxic oxygen

274 consumption rate (MO_2, mg kg^{-1} min^{-1}), was found to increase exponentially with temperature

275 ($R^2 = 0.95$, p<0.001), and was equivalent to 1.2±0.0, 2.3±0.1 and 3.8±0.2 mg kg^{-1} min^{-1} at 6, 12

276 and 18 °C, respectively (Fig. 3B). Q_{10} for temperatures ranging from 6 to 18 °C was found to

277 be 2.7. The exponential relationship between temperature and MO_2 was modeled as

278

279 $$MO_2=0.6564e^{0.0977T}$$

280

281 The limiting oxygen concentration (LOC) was found to increase exponentially with

282 temperature ($R^2=0.80$, $p<0.001$) with LOC equivalent to 2.9±0.1, 3.4±0.1 and 4.3±0.2 at 6, 12

283 and 18 °C, respectively (Fig. 3C). The Q_{10}-value for the increase in LOC with temperature

284 was 1.37, and LOC was modeled as

285

286 $$LOC= 2.3812e^{0.0314T}$$

287

288 During the time period needed to reduce DO from normoxic levels to LOC (1.5-4.5 h),

289 MO_2 was generally found to decline. The time used to reduce DO to LOC, and the reduction

290 in MO_2 within this period is presented in Table 2. Two out of 12 LOC measurements were

291 excluded due to non-significant Davies tests, and in one case, no breakpoint could be

292 estimated.

293

294 *3.2. The relationship between MO_2 and LOS*

295 A strong correlation between MO_2 and LOC was found ($R^2=0.86$, $p<0.001$, Fig. 4A).

296 The recalculation of LOC into LOS (limiting oxygen saturation, expressed as % of air

297 saturation) (Fig. 4B) reduced residual error, increasing R^2 to 0.93 ($p<0.001$, Fig. 4B). In order

298 to determine whether LOS can be determined by MO_2, across experimental temperature and –

299 conditions, data from the present study was compared with data from Barnes et al. (2011),

300 using ANCOVA. Results from Barnes et al. (2011) were found to yield slightly lower LOS

301   for a given MO_2 (p=0.004), equivalent to a 3-6% reduction (in oxygen saturation units) within

302   the range of MO_2 overlap (2.4-4.2 mg kg^{-1} min^{-1}). The relationship between MO_2 and LOS in

303   the present study was determined as

304

305   LOS =9.785MO_2+17.873

306

307   *3.3. The effect of hypoxia acclimation on feed intake, MO_2 and LOC*

308   In Exp II, the feed intake of post-smolts during the meal preceding LOC

309   measurements was reduced according to the DO in hypoxic periods (R^2=0.46, p<0.05), and

310   was equivalent to a 20% reduction in FI of fish from the 80:50 group compared to the control

311   (Fig. 5A). A tendency for increased MO_2 with DO in hypoxic periods was observed, but not

312   statistically significant (R^2=0.31, p=0.068) (Fig. 5B). The linear trend-line suggest that MO_2

313   was reduced by 10% when DO was reduced from 80% to 50% O_2 in hypoxic periods. The

314   MO_2 calculated when DO had been reduced to LOC, showed that the difference in MO_2

315   between groups had been reduced to none at this point of time (R^2=0.01, p=0.749). No effect

316   of hypoxia acclimation status on the limiting oxygen concentration was found (R^2=0.01,

317   p>0.90) (Fig. 5C). The overall LOC was 3.76±0.06.

318

319   *3.4. The combined effect of temperature and oxygen on Vf*

320   The ventilation frequency (Vf) increased both with temperature and declining oxygen

321   levels, until LOC_{Vf} was reached. Below LOC_{Vf}, Vf declined (Fig. 6A). No significant

322   differences between LOC and LOC_{Vf} were found at any of the temperatures (One-way

323   ANOVA, p>0.05) (Table 2). Both the Vf in normoxia (Vf_{norm}; Vf at 90% of air saturation)

324   and at LOC_{Vf} (Vf_{max}) were closely related to MO_2, and data were fitted with second-order

325   polynomial relationships (R^2=0.98 and 0.99) (Fig. 6B). For data obtained at 18 °C, the

326 polynomial curve suggest that Vf_{max} reaches a plateau at MO_2 equal to 4.2 mg kg^{-1} min^{-1} (Fig.

327 6B).

328

329 **4. Discussion**

330 *4.1. The relationship between temperature, MO_2 and LOC*

331   The results from the present experiment show a clear exponential relationship between

332 temperature and oxygen consumption rate (MO_2) of Atlantic salmon post-smolts, similar to

333 what have been found in previous studies on salmonids (Barnes et al., 2011; Brett, 1971; Brett

334 and Glass, 1973). The Q_{10}-value for the entire temperature range (2.7) was somewhat higher

335 than values reported in studies of other, similar-sized salmonids (1.2-2.4) (Brett and Glass,

336 1973; Evans, 1990; Ott et al., 1980), as well as a recent study on Atlantic salmon post-smolts

337 (2.2) (Barnes et al., 2011). The higher Q_{10}-value can be explained by the inclusion of low

338 temperature (6 °C), as the effect of temperature on salmonid metabolism appears to be higher

339 at low temperatures (Evans, 1990; Fivelstad and Smith, 1991; Henry and Houston, 1984).

340 Further, the fish in our study were fed, and as feed intake increases with temperature, the

341 effect of feeding on MO_2 (Forsberg, 1997) can explain the more steep increase in MO_2 with

342 temperature observed in the present study.

343   The measured oxygen consumption rates (MO_2) of post-smolts at 6 and 12 °C were

344 close to what can be obtained for fed post-smolts (500 g) swimming at low speed (0.3 body

345 lengths, BL, s^{-1}), using the model presented by Forsberg (1994). At higher temperatures,

346 observed MO_2 was higher than that predicted by Forsberg's model, which does not predict

347 exponential increase with temperature. The measured MO_2 was also comparable to the lower

348 end of the MO_2 range observed in fed Atlantic salmon post-smolts in a study by Bergheim et

349 al. (1991), as well as to the MO_2 of fasted (>12 h), resting post-smolts found by Wilson et al.

350 (2007). Together, these results suggest that the MO_2 of post-smolts observed in the present

351 study was at the lower end of the range for what can be expected for post-smolts kept under

352 production conditions in sea cages. Both the swimming speed and feed intake of fish were

353 probably at relatively low levels, due to the low water current speed in the tanks and repeated

354 experiences of hypoxic stress (Bernier and Craig, 2005; Remen et al., 2012).

355   Compared to the only other study that has investigated LOC in Atlantic salmon post-

356 smolts (Barnes et al. 2011), LOC was 25% higher in the present experiment, at the

357 temperature common of both studies (18 °C). This is likely to be a result of using fed fish,

358 with higher metabolic rates, compared to the use of fasted fish in the experiment by Barnes et

359 al. (2011), as both studies show a strong, positive correlation between MO_2 and LOC. The

360 plots of limiting oxygen saturation (LOS) values against MO_2, showed that only slight

361 differences in LOS was obtained for a given MO_2 when these two studies were compared,

362 suggesting that relatively good estimates of LOS can be made from MO_2 measurements,

363 across experimental conditions and -temperatures. The stronger linear relationship between

364 MO_2 and LOS ($R^2=0.93$) than between MO_2 and LOC ($R_2=0.86$), suggests that branchial

365 oxygen transfer is more dependent on water oxygen tension than oxygen concentration, at

366 least when DO is reduced to levels around LOC, where gill ventilation is at its maximum.

367 Therefore, assessment of whether Atlantic salmon are provided with DO above their hypoxia

368 tolerance threshold may be more accurate using oxygen saturation than concentration.

369

370 *4.2. Hypoxia tolerance thresholds for post-smolts in production conditions*

371   Using the relationship between MO_2 and LOS presented here, the hypoxia tolerance

372 threshold (LOS) of post-smolts, with metabolic rates as similar to fish under production

373 conditions in sea cages as possible, can be estimated based on previously published MO_2

374 measurements. Several studies have investigated the MO_2 of post-smolts (100-800 g) in large

375 production tanks, or experimental tanks, where fish are fed to satiation, and where swimming

376 activity (~0.4-1.7 body lengths s^{-1}) varies according to the water current speed in the tanks

377 (Atkins and Benfey, 2008; Bergheim et al., 1991; Castro et al., 2011; Fivelstad and Smith,

378 1991; Forsberg, 1994). The resulting LOS values at different temperatures are summarized in

379 Fig. 7. Generally, the estimated LOS values are higher than the LOS obtained in the present

380 study (up to 73% higher), and suggest that the hypoxia tolerance threshold should be

381 increased by at least 40% compared to the LOS obtained in the present study, in order to serve

382 as practical guidelines for Atlantic salmon aquaculture (see Fig. 7). For practical use, it should

383 be emphasized that the hypoxia tolerance threshold represents a threshold for hypoxic stress

384 (Vianen et al., 2001) and compromised survival (Nilsson and Nilsson, 2008), and that

385 negative effects on parameters of production performance (e. g. feeding, digestion and

386 growth) are expected at higher levels of DO (e.g. Remen et al., 2012). Knowledge of both

387 hypoxia tolerance thresholds and thresholds for maintained feeding and growth at various

388 temperatures would enable an approximation of the graded negative effect of hypoxia

389 between these two thresholds. Further, it should be emphasized that in some situations, the

390 hypoxia tolerance threshold can be expected to increase beyond the guideline presented here,

391 for example if fish are subjected to acute stress, (Folkedal et al., 2012), or if forced to swim at

392 high speed (Wilson et al., 2007). The relationships between temperature, MO$_2$ and LOS

393 should be validated for larger Atlantic salmon (> 800 g) in the sea water phase and

394 investigated further for higher temperatures.

395

396 *4.3. Ventilation frequency as an indicator of MO$_2$*

397   The increasing ventilation frequency (Vf$_{norm}$) with temperature in Atlantic salmon

398 agreed with Millidine et al. (2008), and the increasing, and then decreasing Vf as oxygen

399 declined towards, and then below LOC, is in agreement with previous observations in a range

400 of teleosts (see Perry et al., 2009, for review). Our results support Millidine et al. (2008) in

401   that Vf is a good indicator of MO_2 in normoxic conditions, but not during reductions in

402   oxygen, due to the increasing Vf, and relatively stable MO_2 as oxygen declines towards LOC.

403

404   *4.4. The effect of acclimation to periodic hypoxia on MO_2 and LOC*

405   In fish acclimated to cyclic hypoxia, the tendency for reduced MO_2 with reduced DO

406   in hypoxic periods (~10% reduction) corresponded well with the reduction in MO_2 that can be

407   expected from the 20% reduction in feed intake, using a model presented by Forsberg et al.,

408   1997) for adult Atlantic salmon. Their results suggest that MO_2 is reduced by 10% when

409   ration is reduced by 20%. Our results are in line with previous rapports of reduced MO_2 as a

410   consequence of reduced feed intake in turbot, European sea bass and Atlantic cod subjected to

411   hypoxia (Claireaux et al., 2000; Pichavant et al., 2000; 2001), although marginal in the

412   present experiment. Feeding fish in normoxic periods are likely to have reduced the negative

413   effect of periodic hypoxia on feed intake and MO_2, compared to experiments where fish are

414   fed in hypoxia (e.g. Remen et al., 2012). The tendency for reduced MO_2 did not result in

415   lowered LOC. This is in line with previous findings in Atlantic cod (Peterson and Gamperl,

416   2010, 2011) and Atlantic salmon post-smolts (Remen et al., 2012), and suggest that frequent

417   exposure to severe hypoxia does not reduce the hypoxia sensitivity of Atlantic salmon.

418

419   *4.5. Conclusions and perspectives*

420   In conclusion, the limiting oxygen concentration (LOC) of Atlantic salmon post-

421   smolts was found to increase exponentially for temperatures ranging from 6 to 18 °C, and a

422   close correlation between the oxygen consumption rate (MO_2) and the limiting oxygen

423   saturation (LOS) was found within and across temperatures. LOS values for different

424   temperatures found in the present experiment are likely to be at the lower end of the LOS

425   continuum that can be expected at a given temperature in a sea cage, and may be implemented

426 by the salmon farming industry as hypoxia tolerance thresholds for fish with relatively low

427 metabolic rates (e.g. when feeding ration is restricted). Hypoxia tolerance thresholds for fish

428 with higher metabolic rates were estimated. Acclimation to hypoxia did not lower LOC,

429 suggesting that negative effects of severe hypoxia are not reduced as a result of physiological

430 adjustments.

431

436

437 **References**

438 Atkins, M.E., Benfey, T.J., 2008. Effect of acclimation temperature on routine metabolic rate

439 　in triploid salmonids. Comp. Biochem. Physiol. A- Mol. Integr. Physiol. 149, 157-161.

440 Barnes, R., King, H., Carter, C.G., 2011. Hypoxia tolerance and oxygen regulation in Atlantic

441 　salmon, *Salmo salar* from a Tasmanian population. Aquaculture 318, 397-401.

442 Bergheim, A., Seymor, E.A., Sanni, S., Tyvold, T., 1991. Measurements of oxygen

443 　consumption and ammonia excretion of Atlantic salmon (*Salmo salar* L.) in commercial-

444 　scale, single-pass freshwater and seawater landbased culture systems. Aquac. Eng. 10, 251-

445 　267.

446 Bernier, N.J., Craig, P.M., 2005. CRF-related peptides contribute to stress response and

447 　regulation of appetite in hypoxic rainbow trout. Am. J. Phys. - Reg. Int. Comp. Physiol.

448 　289, 982-990.

449   Brett, J.R., 1971. Energetic responses of salmon to temperature - study of some thermal

450   relations in physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*)

451   Am. Zool. 11, 99-113.

452   Brett J.R., and Glass, N.R., 1973. Metabolic responses and critical swimming speeds of

453   sockeye salmon (*Onorhynchus nerka*) in relation to size and temperature. J. Fish. Res.

454   Board. Can. 30, 379-387.

455   Brett, J.R., Groves, T.T.D., 1979. Physiological energetic, in: Hoar, W.S., Randall, D.J., Brett,

456   J.R. (Eds.), Fish Physiology, vol. 8. Academic Press, New York, pp. 280–352.

457   Burt, K., Hamoutene, D., Mabrouk, G., Lang, C., Puestow, T., Drover, D., Losier, R., Page,

458   F., 2012. Environmental conditions and occurrence of hypoxia within production cages of

459   Atlantic salmon on the south coast of Newfoundland. Aquac. Res. 43, 607-620.

460   Burton, D.T., Heath, A.G., 1980. Ambient oxygen tension (P_{O2}) and transition to anaerobic

461   metabolism in O_2 three species of freshwater fish. Can. J. Fish. Aquat. Sci. 37, 1216–1224.

462   Castro, V., Grisdale-Helland, B., Helland, S.J., Kristensen, T., Jorgensen, S.M., Helgerud, J.,

463   Claireaux, G., Farrell, A.P., Krasnov, A., Takle, H., 2011. Aerobic training stimulates

464   growth and promotes disease resistance in Atlantic salmon (*Salmo salar*). Comp. Biochem.

465   Physiol. A -Mol. Integr. Physiol. 160, 278-290.

466   Claireaux, G., Webber, D.M., Lagardere, J.P., Kerr, S.R., 2000. Influence of water

467   temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus*

468   *morhua*). J. Sea Res. 44, 257-265.

469   Crampton, V., Hølland, P.M., Bergheim, A., Gausen, M., Næss, A., 2003. Oxygen effect on

470   caged salmon. Fish Farming International- June edition, 26-27.

471   Davis, J.C., 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on

472   canadian species: a review. J. Fish. Res. Board Can 32, 2295-2332.

473   Elliott, J., Elliott, J., 2010. Temperature requirements of Atlantic salmon, *Salmo salar*, brown

474   trout, *Salmo trutta,* and Arctic charr, *Salvelinus alpinus*: predicting the effects of climate

475   change. J. Fish. Biol. 77, 1793-1817.

476   Evans, D.O., 1990. Metabolic compensation by rainbow trout: effects on standard metabolic

477   rate and potential usable power. Trans. Am. Fish. Soc. 119, 585-600.

478   Farrell, A. P., Eliason, E., Sandblom, E., Clark, T.D., 2009. Fish cardiorespiratory physiology

479   in an era of climate change. Can. J. Zool. 87, 835-851.

480   Farrell, A.P., Richards, J.G., 2009. Defining hypoxia: an integrative synthesis of the responses

481   of fish to hypoxia, in: Richards, J.G., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology,

482   vol. 27. Academic Press, New York, pp. 487-503.

483   Fivelstad, S., Smith, M.J., 1991. The oxygen consumption rate of Atlantic salmon (*Salmo*

484   *salar* L.) reared in a single pass landbased seawater system. Aquacult. Eng. 10, 227-235.

485   Folkedal, O., Torgersen, T., Olsen, R.E., Fernø, A., Nilsson, J., Oppedal, F., Stien, L.H.,

486   Kristiansen, T.S., 2012. Duration of effects of acute environmental changes on food

487   anticipatory behaviour, feed intake, oxygen consumption, and cortisol release in Atlantic

488   salmon parr. Physiol. Behav. 105, 283-291.

489   Forsberg, O.I., 1994. Modeling oxygen consumption rates of post-smolt Atlantic salmon in

490   commercial-scale, land-based farms. Aquacult. Int. 2, 180-196.

491   Forsberg, O.I., 1997. The impact of varying feeding regimes on oxygen consumption and

492   excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon *Salmo salar* L.

493   Aquac. Res 28, 29-41.

494   Fry, F. E. J., 1947. Effects of the environment on animal activity. University of Toronto

495   Studies in Biological Series 55. Publ. Ont. Fish. Res. Lab. 68, 5-62.

496 Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish, in: Hoar,

497 W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 6: Environmental Relations and

498 Behaviour. Academic Press, London/New York. pp. 1-99.

499 Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement

500 of daily feed intake of groups of fish in tanks. Aquaculture 139, 157-163.

501 Henry, J. A. C., Houston, A. H., 1984. Absence of respiratory acclimation to diurnally-cycling

502 temperature conditions in rainbow trout. Comp. Biochem. Physiol. A- Physiol. 77, 727-

503 734.

504 Houde, E.D., Scheckter, R.C., 1981. Growth rates, rations and cohort consumptions of marine

505 fish larvae in relation to prey concentration. Rapp. P.-V. Re`un. - Cons. Int. Explor. Mer

506 178, 441– 453.

507 Johansson, D., Ruohonen, K., Kiessling, A., Oppedal, F., Stiansen, J.E., Kelly, M., Juell, J.E.,

508 2006. Effect of environmental factors on swimming depth preferences of Atlantic salmon

509 (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord

510 site. Aquaculture 254, 594-605.

511 Johansson, D., Juell, J.E., Oppedal, F., Stiansen, J.E., Ruohonen, K., 2007. The influence of

512 the pycnocline and cage resistance on current flow, oxygen flux and swimming behaviour

513 of Atlantic salmon (*Salmo salar* L.) in production cages. Aquaculture 265, 271-287.

514 Kutty, M.N., Saunders, R.L., 1973. Swimming performance of young Atlantic salmon (*Salmo*

515 *salar*) as affected by reduced ambient oxygen concentration. J. Fish. Res. Board Can. 30,

516 223-227.

517 Lai, J.C.C., Kakuta, I., Mok, H.O.L., Rummer, J.L., Randall, D., 2006. Effects of moderate

518 and substantial hypoxia on erythropoietin levels in rainbow trout kidney and spleen. J. Exp.

519 Biol. 209, 2734-2738.

520   Mark, F.C., Bock, C., Pörtner, H.O., 2002. Oxygen-limited thermal tolerance in Antarctic fish

521   investigated by MRI and ^{31}P-MRS. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283,

522   1254–1262.

523   Millidine, K., Metcalfe, N., Armstrong, J., 2008. The use of ventilation frequency as an

524   accurate indicator of metabolic rate in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish.

525   Aquat. Sci. 65, 2081-2087.

526   Muggeo, V.M.R., 2003. Estimating regression models with unknown breakpoints. Stat. Med.

527   22, 3055-3071.

528   Muggeo, V.M.R., 2008. Segmented: An R-package to fit regression models with broken-line

529   relationships. R-News vol. 8/1, 20-25. http://www.r-project.org/doc/Rnews/Rnews_2008-

530   1.pdf.

531   Neill, W.H., Bryan, J.D., 1991. Responses of fish to temperature and oxygen, and response

532   integration through metabolic scope. J. World Aquacult. Soc. 3, 31-57.

533   Nilsson, G.E., Östlund-Nilsson, S., 2008. Does size matter for hypoxia tolerance in fish? Biol.

534   Rev. 83, 173-189.

535   Oppedal, F., Juell, J.-E., Johansson, D., 2007. Thermo- and photoregulatory swimming

536   behaviour of caged Atlantic salmon: implications for photoperiod management and fish

537   welfare. Aquaculture 265, 70–81.

538   Oppedal, F., Dempster, T., Stien, L., 2011. Environmental drivers of Atlantic salmon

539   behaviour in sea-cages: a review. Aquaculture 311, 1-18.

540   Ott, M.E., Heisler, N., Ultsch, G.R., 1980. A re-evaluation of the relationship between

541   temperature and oxygen the critical oxygen tension in freshwater fishes. Comp. Biochem.

542   Physiol. 67A, 337-340.

543   Perry, S., Reid, S., 1994. The effects of acclimation temperature on the dynamics of

544   catecholamine release during acute hypoxia in the rainbow trout *Oncorhynchus mykiss*. J.

545   Exp. Biol. 186, 289-307.

546   Perry, S.F., Jonz, M.G., Gilmour, K.M., 2009. Oxygen sensing and the hypoxic ventilatory

547   response, in: Richards, J.G., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology vol. 27:

548   Hypoxia. Elsevier, London, pp. 193-253.

549   Peterson, L.H., Gamperl, A.K., 2010. Effects of acute and chronic hypoxia on the swimming

550   performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). J.

551   Exp. Biol. 213, 808-819.

552   Peterson, L.H., Gamperl, A.K., 2011. Cod (*Gadus morhua*) cardiorespiratory physiology and

553   hypoxia tolerance following acclimation to low-oxygen conditions. Physiol. Biochem.

554   Zool. 84, 18-31.

555   Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A., Quemener, L.,

556   Maxime, V., Nonnotte, G., Boeuf, G., 2000. Effects of hypoxia on growth and metabolism

557   of juvenile turbot. Aquaculture 188, 103-114.

558   Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A., Boeuf, G., 2001.

559   Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in

560   juvenile turbot and European sea bass. J. Fish Biol. 59, 875-883.

561   Pörtner, H.O., 2010. Oxygen-and capacity-limitation of thermal tolerance: a matrix for

562   integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881-

563   893.

564   Remen, M., Oppedal, F., Torgersen, T., Imsland, A.K., Olsen, R.E., 2012. Effects of cyclic

565   environmental hypoxia on physiology and feed intake of post-smolt Atlantic salmon:

566   Initial responses and acclimation. Aquaculture 326-329, 148-155.

567 Schmidt-Nielsen, K., 1997. Animal physiology, adaptation and environment, 5th ed.

568   Cambridge University Press, Cambridge, UK, 595 pp.

569 Soivio, A., Nikinmaa, M., Westman, K., 1980. The blood oxygen binding properties of

570   hypoxic *Salmo gairdneri*. J. Comp. Physiol. B 136, 83-87.

571 Tetens, V., Lykkeboe, G., 1981. Blood respiratory properties of rainbow trout, *Salmo*

572   *gairdneri*: responses to hypoxia acclimation and anoxic incubation of blood in vitro. J.

573   Comp. Physiol. B 145, 117-125.

574 Thorarensen, H., Farrell, A.P., 2011. The biological requirements for post-smolt Atlantic

575   salmon in closed-containment systems. Aquaculture 312, 1-14.

576 Van Raaij, M.T.M., Pit, D.S.S., Balm, P.H.M., Steffens, A.B., Van den Thillart, G., 1996.

577   Behavioral strategy and the physiological stress response in rainbow trout exposed to

578   severe hypoxia. Horm. Behav. 30, 85-92.

579 Vianen, G.J., Van den Thillart, G.E.E.J.M., Van Kampen, M., Van Heel, T.I., Steffens, A.B.,

580   2001. Plasma lactate and stress hormones in common carp (*Cyprinus carpio*) and rainbow

581   trout (*Oncorhynchus mykiss*) during stepwise decreasing oxygen levels. Neth. J. Zool. 51,

582   33-50.

583 Wang, T., Lefevre, S., Huong, D.T.T., Van Cong, N., Bayley, M., 2009. The effects of

584   hypoxia on growth and digestion, in: Farrell, A.P., Brauner, C.J., Richards, J.G. (Eds.),

585   Fish physiology vol. 27: Hypoxia. Elsevier, London, pp. 361-396.

586 Wilson, C., Friesen, E., Higgs, D., Farrell, A., 2007. The effect of dietary lipid and protein

587   source on the swimming performance, recovery ability and oxygen consumption of

588   Atlantic salmon (*Salmo salar*). Aquaculture 273, 687-699.

589

590

591 **Tables**

592

593 **Table 1.** Fish weights (g), lengths (cm, fork length) and condition factors (CF) of Atlantic salmon post-smolts,

594 measured on days -48 and 46 after first change of temperature (day 0) in Experiment I, and on the day following

595 LOC measurements in Experiment II. The number of replicate fish tanks and total number of fish measured per

596 treatment are provided. For Experiment II, group names indicate the oxygen saturation in normoxic and hypoxic

597 periods, respectively.

	Day	Group	Weigth (g)	Length (cm)	CF	Replicate tanks	Total number of fish
Exp I	-48		291±4	28.2±0.09	1.29±0.01	4	137
	46		513±10	34.6±0.21	1.24±0.01		
Exp II	34	80:50	569±15	36.0±0.3	1.20±0.01	3	293
	34	80:60	576±3	36.3±0.1	1.18±0.02	3	294
	34	80:70	578±9	36.2±0.2	1.20±0.01	3	294
	34	80:80	608±18	36.8±0.3	1.20±0.01	3	270

598

599　**Table 2.** The time used to reduce the dissolved oxygen concentration (DO) from normoxic levels to LOC

600　(duration; minutes), the decline in oxygen consumption rates (MO_2) during this time period (MO_2 decline; % of

601　normoxic levels) and the limiting oxygen concentration for increased ventilation frequency (LOC_{Vf}, mg L^{-1}) at

602　the different temperatures in Experiment I, and in fish acclimated to periodic hypoxia of varying severity (group

603　names indicate oxygen saturation in normoxic and hypoxic periods) at 16 °C for 33 days in Experiment II. The

604　number of verified observations is indicated by number of replicate tanks (N). NA= not applicable.

	T (°C)	Group	Duration (min)	MO_2 decline (%)	LOC_{Vf} (mg L^{-1})	N (replicate tanks)
	6	NA	252±9	1±6	2.4±0.4	3
Exp I	12	NA	167±0	6±3	3.0±0.1	3
	18	NA	99±2	10±4	5.1±0.2	4
	16	80:80	236±12	5±2	NA	3
	16	80:70	266±23	6±2	NA	3
Exp II	16	80:60	271±12	11±4	NA	3
	16	80:50	228±3	19±8	NA	3

605

606

607   **Figure captions**

608   **Fig. 1.** Schematic overview over the daily fluctuations in oxygen concentration (mg L^{-1}) in the four

609   experimental groups during the acclimation period (33 days) preceding LOC measurements in Exp II. Shaded

610   areas represent feeding periods.

611   **Fig. 2 A-B.** Examples of (A) measured oxygen consumption rates (MO$_2$, mg kg^{-1} min^{-1}) and (B) gill ventilation

612   frequencies (Vf, gill movements s^{-1}) of Atlantic salmon post-smolts plotted against the dissolved oxygen

613   concentration (DO, mg L^{-1}) in one replicate tank during a progressive decline in DO. A segmented regression

614   model was fitted to the plot of MO$_2$ against DO and used to estimate the limiting oxygen concentration (LOC). A

615   third order polynomial function was fitted to the plot of Vf against DO and used to estimate Vf in normoxia (8.7

616   mg L^{-1}, termed Vf$_{norm}$), the LOC for compensatory gill ventilation (LOC$_{Vf}$) and the maximum ventilation

617   frequency (Vf$_{max}$). The data presented are from fish acclimated to 12 °C and fed to satiation ~2 h prior to the

618   progressive reduction in DO (Exp I).

619   **Fig. 3 A-C.** The effect of temperature (°C) on A) the feed intake (FI, % of biomass) of Atlantic salmon post-

620   smolts during the morning meal preceding LOC measurements, B) the normoxic oxygen consumption rate

621   (MO$_2$, mg kg^{-1} min^{-1}) and C) the limiting oxygen concentration (LOC) of undisturbed Atlantic salmon post-

622   smolts fed to satiation. A logarithmic curve was fitted to the data in A, while exponential curves were fitted to

623   the data in B and C.

624

625   **Fig. 4 A-B.** A) The limiting oxygen concentration (LOC, mg L^{-1}) and B) the corresponding limiting oxygen

626   saturation (LOS, % of air saturation) of Atlantic salmon post-smolts in response to oxygen consumption rates

627   (MO$_2$, mg kg^{-1} min^{-1}) at different temperatures (see legend), obtained in the present study (filled symbols) and a

628   study of Barnes et al. (2011) (open symbols). Linear regression lines were fitted to both data sets, and multiple

629   R^2 are shown in the figures.

630

631   **Fig. 5.** The effect of hypoxia severity on A) the feed intake (FI, % of biomass) ingested during the meal

632   preceding LOC measurement), B) the oxygen consumption rate (MO$_2$, mg kg^{-1} min^{-1}) and C) the limiting oxygen

633   concentration (LOC, mg L^{-1}) of Atlantic salmon post-smolts subjected to cyclic hypoxia (~2 h every 6 h, DO=

634   5.6, 4.8 or 4.0 mg L^{-1}, control kept at constant 6.4 mg L^{-1}) for 33 days. Post-smolts were held at 16 °C and

635   continuous lighting, and feed was provided in normoxic periods.

636

637   **Fig. 6.** The data presented in A) show the effect of oxygen (DO, mg L^{-1}) on the gill ventilation frequency (Vf,

638   movements s^{-1}) of Atlantic salmon post-smolts at 6, 12 and 18 °C. Temperature is indicated by the use of

639   different symbol shapes (see legend). Data points represent the average Vf (±SEM) for DO at intervals

640   corresponding to 10% of air saturation, calculated from the polynomial relationships between DO and Vf

641   obtained for each replicate tank (n=4). The data presented in B) show the relationships between oxygen

642   consumption rate (MO_2, mg kg^{-1} min^{-1}) and the normoxic Vf (Vf_{norm}; filled symbols, DO equivalent to 90% of air

643   saturation) and the maximal Vf (Vf_{max}; open symbols, DO equivalent to the limiting oxygen concentration for

644   compensatory gill ventilation; LOC_{Vf}). Temperature is indicated by the use of different symbol shapes (see

645   legend).

646

647   **Fig. 7.** The limiting oxygen saturation (LOS, % of air saturation) of fed Atlantic salmon post-smolts at

648   temperatures ranging from 6-18 °C obtained in the present study (black line), compared to LOS estimated from

649   MO_2 measured in other studies of Atlantic salmon smolts/post-smolts fed to satiation and swimming at various

650   speed (~0.4-1.7 body lengths, BL, s^{-1}) (broken lines). LOS was estimated using $LOC=9.785*MO_2+17.873$.

651   Based on this comparison, it is suggested that LOS should be increased by at least 40% (grey line) in order to

652   serve as a practical guideline for the salmon farming industry. The number next to broken lines indicate the

653   literature reference; 1) Fivelstad and Smith, 1991 (200-800 g post-smolts, swimming speed ~0.4-0.7 body

654   lengths, BL, s^{-1}), 2) Bergheim, 1991 (520-800 g post-smolts, ~1.2-1.7 BL s^{-1}), 3) Castro et al., 2011 (100-200 g

655   post-smolts, 0.7 BL s^{-1}), 4) Forsberg, 1994 (500 g post-smolts, 1 BL s^{-1}) and 5) Atkins and Benfey, 2008 (~150
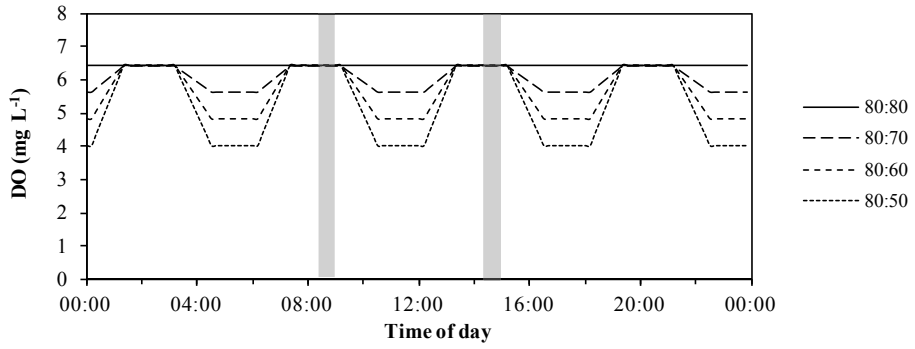
656   g parr/smolts, swimming speed unknown).

657

658

Figures

Fig. 1
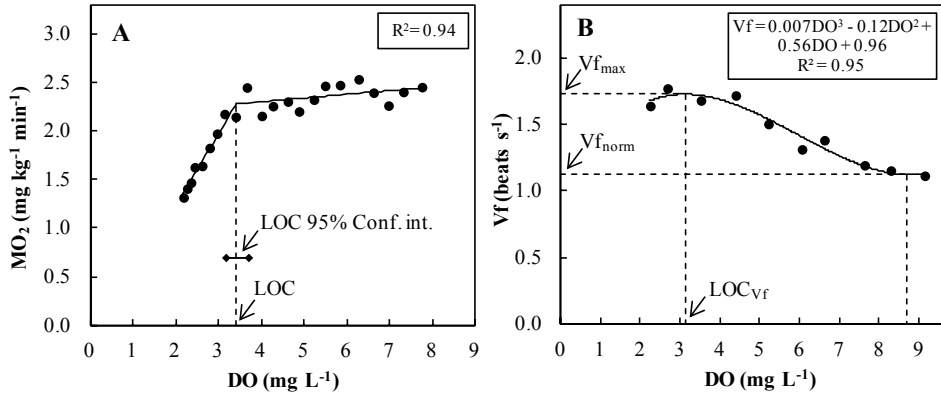

662

663

664

665   **Fig. 2 A-B**
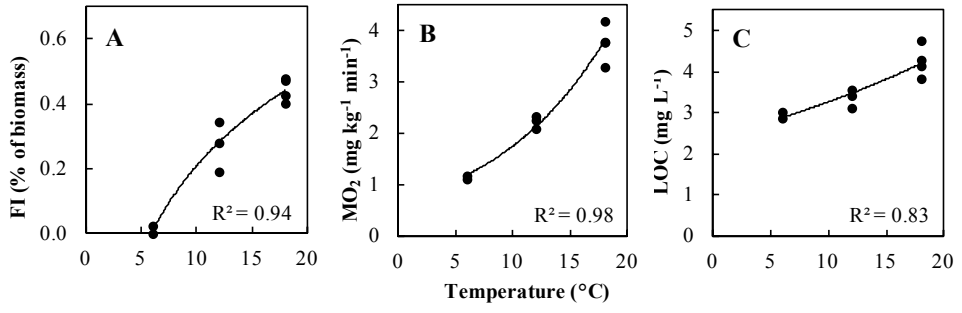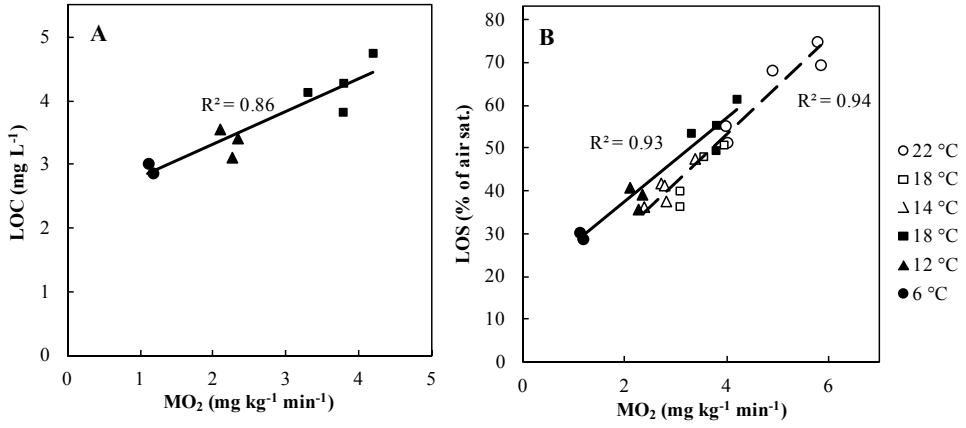

666
667

668
669

670 **Fig. 3 A-C**

671

672

673   **Fig. 4 A-B**

674

675

676 **Fig. 5 A-C**
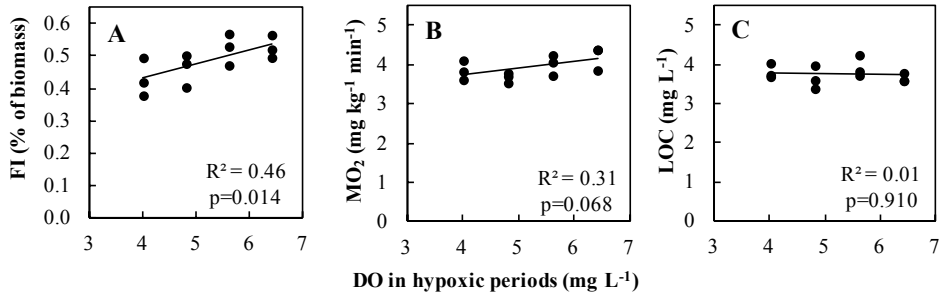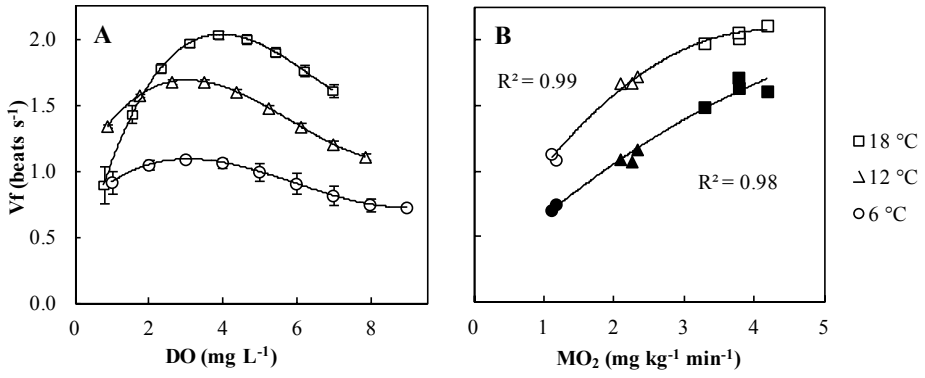677

678
679

680   **Fig. 6 A-B**

681

682

683 **Fig. 7**

684