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Complete List of Authors:	Prinet, Aurélie; Université du Québec à Rimouski, Institut des sciences de la mer de Rimouski Vagner, Marie; UMR LIENSs 7266, Institut du Littoral et de l'Environnement Chabot, Denis; Pêches et Océans Canada, Institut Maurice Lamontagne Audet, Céline; Université du Québec à Rimouski, Institut des sciences de la mer de Rimouski
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Impact of hypoxia on the metabolism of Greenland halibut (*Reinhardtius hippoglossoides*)

Aurélie Dupont-Prinet[1], Marie Vagner[1,3], Denis Chabot[2] and Céline Audet[1]

[1] Institut des Sciences de la Mer – Université du Québec à Rimouski, 310, allée des Ursulines, Rimouski, QC, G5L 3A1, Canada

[2] Institut Maurice Lamontagne, Pêches et Océans Canada, 850 route de la Mer, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada

[3] Present address: UMR LIENSs 7266, Institut du Littoral et de l'Environnement, 2 rue Olympe de Gouges 17000 La Rochelle, France

Author for correspondence:
Aurélie Dupont-Prinet
Institut des sciences de la mer de Rimouski (ISMER)
Université du Québec à Rimouski (UQAR)
310, allée des Ursulines
Rimouski (Québec)
G5L 3A1
Fax (418) 724-1842
Aurelie.Dupont-Prinet@uqar.qc.ca

Abstract

Greenland halibut, especially juveniles, are frequently found in severely hypoxic areas (18–25% saturation) of the St. Lawrence Estuary. We investigated the tolerance of this species to hypoxia and evaluated the consequences of low oxygen levels on metabolic capacity. At 5°C, juveniles had a higher critical oxygen threshold than adults (15 *vs* 11% saturation), indicating that they were less tolerant to hypoxia. Severe hypoxia (19% saturation) did not affect the juveniles' standard metabolic rate but significantly reduced (by 55%) their maximum metabolic rate compared to normoxia. Consequently, the aerobic scope was reduced by 72% in hypoxia compared to normoxia. In juveniles, severe hypoxia increased the duration of digestive processes. The decrease in aerobic scope in hypoxia and the determination of O_{2crit} at a saturation level close to actual field DO values strongly suggest that juveniles from the St. Lawrence Estuary are living at the edge of their metabolic capacity. Consequently, the growth and distribution of Greenland halibut could be affected if there are further declines in dissolved oxygen availability.

Key words: aerobic scope, specific dynamic action, standard metabolic rate, critical oxygen threshold.

Résumé

Les flétans du Groenland, en particulier les juvéniles, sont fréquemment pêchés dans les zones hypoxiques (18–25 % saturation) de l'Estuaire du St. Laurent. L'objectif de cette étude était d'évaluer la tolérance à l'hypoxie chez cette espèce ainsi que les conséquences des faibles niveaux d'oxygène sur sa capacité métabolique. À 5°C, les juvéniles ont un seuil critique d'oxygène supérieur à celui des adultes (15 *vs* 11 % saturation), indiquant qu'ils sont moins tolérants à l'hypoxie. L'hypoxie sévère (19 % saturation) n'a pas affecté le taux métabolique standard des juvéniles, mais a réduit significativement (de 55 %) leur taux métabolique maximal par rapport à la normoxie. Par conséquent, le registre aérobie a été réduit de 72 % en hypoxie par rapport à la normoxie. Chez les juvéniles, l'hypoxie sévère augmente la durée du processus de digestion. La réduction du registre aérobie en hypoxie et la détermination du seuil critique d'oxygène à des niveaux près de ceux actuellement présents dans l'estuaire du Saint-Laurent suggèrent que les juvéniles ont peu de marge de manœuvre sur le plan métabolique. Conséquemment, toute nouvelle dégradation des conditions d'oxygénation pourrait affecter la croissance et la distribution du flétan du Groenland.

Introduction

Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), is an important commercial species in the North Atlantic, and for the last 20 years it has been the most important commercial demersal fish in the Estuary and Gulf of St. Lawrence (EGSL), accounting for 58% of fish catches in terms of biomass (DFO 2011). Recently, Ait Youcef et al. (2013) have shown that the St. Lawrence estuary is the major nursery area for the EGSL population and that habitats selected by Greenland halibut are characterized by low dissolved oxygen (DO) levels. The strong association between higher fish densities and the low DO concentrations suggest a high tolerance of Greenland halibut to hypoxia.

Between 1930 and the early 1980s, DO levels decreased by half in the deep water of the St. Lawrence Estuary due to an increase in the proportion of warm, oxygen-poor North Atlantic central water coming into the system and an increase in organic matter flow (Coote and Yeats 1979; Gilbert et al. 2005, 2007; Thibodeau et al. 2006, 2010; Genovesi et al. 2011). Since the mid-1980s, DO levels in water deeper than 150 m in the St. Lawrence Estuary have been stable at around 18–25% saturation (sat. hereafter) (Gilbert et al. 2007). However, climate change and increasing human population have the potential to further accentuate hypoxia in the St. Lawrence system.

Hypoxia in estuaries and coastal waters is known to affect biodiversity (Breitburg 2002; Switzer et al. 2009; Zhang et al. 2010*a*). Habitat suitability, behaviour, and physiology could all be affected, and effects vary according to species, life history stage, and predator–prey interactions among others (Breitburg 2002; Eby et al. 2005; Switzer et al. 2009; Brandt et al. 2011). If we consider physiological effects and fish performance, the DO level directly impacts metabolism (Fry 1971; Brett 1979) and, consequently, growth, activity level, and the ability to process meals (Stewart et al. 1967; Andrews et al. 1973; Weber and Kramer 1983; Cech et al. 1984; Pedersen 1987; Van den Thillart et al. 1994; Chabot and Dutil 1999; Dupont-Prinet et al. 2009; Zhang et al. 2010*b*). In the context of global changes, climate change may directly impact water temperature, which will impact hypoxia events (frequency and duration) and the metabolic performance of fishes (Pörtner and Peck 2010). From the 1930s to the 1980s, the bottom waters of the St. Lawrence Estuary warmed by 1.65°C (Gilbert et al. 2005); this increased bacterial metabolism, which played a role in DO decrease (Genovesi et al. 2011). Because metabolic rates directly scale with temperature in exothermic organisms, the increase in metabolism associated with climate warming could reduce the body size of ectotherms unless organisms can compensate with greater food intake or the reallocation of caloric resources (e.g., Sheridan and Bickford 2011). In modifying the capacity-limitation of a species, climate change makes the species more vulnerable and has a direct impact on productivity, food webs, and biodiversity (Pörtner 2010). To determine how these changes will impact specific environments represents huge scientific challenges, and one of the first steps to achieving this is to understand how each species tolerates or may adapt to hypoxic conditions.

The aim of this study was to determine the tolerance of Greenland halibut to hypoxia and to evaluate the consequences of low DO levels on metabolic capacity. To do so, we determined the standard metabolic rate (SMR) and the critical oxygen threshold (O_{2crit}) in both juvenile and adult Greenland halibut. The maximum metabolic rate (MMR) and the specific dynamic action (SDA; the transient post-prandial increase in metabolic rate) were also investigated in juveniles under severely hypoxic conditions.

Materials and Methods

Experimental animals

Adult Greenland halibut were caught by long-line fishermen near Rivière-au-Renard (QC, Canada) whereas juveniles were caught by trawling during Fisheries and Oceans Canada (DFO) fishing operations in the St. Lawrence Estuary. Fish were held for several months under a natural photoperiod at the Maurice Lamontagne Institute (Mont-Joli, QC, Canada) in rearing tanks supplied with natural seawater (salinity ~28; DO ~100% sat.**)** maintained at 5°C. Animals were fed three times a week to satiation with capelin (*Mallotus villosus*) and shrimp (*Pandalus* sp.). Fish fasted for one week before all experiments. However, for the SDA experiments, fasted fish were force-fed 15 min before being introduced into the respirometer (see below). Prior to any experiment, Stress Coat® (0.26 $mL\cdot L^{-1}$; Mars Fishcare North America Inc., PA, USA) was added to the water to reduce mucus loss in fish.

Experimental methods complied with the regulations of the Canadian Council on Animal Care and were approved by the Maurice Lamontagne Institute and the Université du Québec à Rimouski animal care committees.

Experimental setup

Five respirometers (48.77 L for adults; 2.18 L for juveniles) were placed in a 1359 L rearing tank provided with a constant flow of aerated seawater at 5°C. Incoming water and recycled water from the tank flowed through a gas-exchange column. Oxygen partial pressure (PO_2) was continuously recorded using a regulator system (Oxy-Reg, Loligo Systems, Denmark; Mini DO galvanic probe, OxyGuard International A/S, Denmark). Nitrogen was injected into the column through a solenoid valve as needed to keep DO within the desired range. When normoxia was desired, air was continuously injected into the column. Oxygen pressure was converted to percent saturation relative to the pressure of oxygen in the air at standard atmospheric pressure after correcting for vapour pressure at 5°C and salinity of 28. The tank containing the respirometers was isolated in a room kept dark at night and in low light in daytime (red lights, with some white light penetrating from surrounding labs). The tank was shielded with opaque curtains to further prevent visual disturbance of the fish.

Oxygen uptake ($\dot{M}O_2$, in mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) in each respirometer was measured by intermittent-flow respirometry (Steffensen 1989): respirometers were flushed with normoxic or hypoxic water for 5 min and closed for 15 min. This cycle was repeated for as long as the fish were in the respirometers. The linear decline in DO observed during the last 13 min was used to calculate $\dot{M}O_2$ according to equation 2 of Steffensen (1989) and equation 8 of Garcia and Gordon (1992) for oxygen solubility. DO was monitored every second using a fibre optic oxygen meter (one-channel Fibox 3 or four-channel Oxy-4 Mini, PreSens, Germany) connected to a Daq 1 or Daq 4 automated control system associated with the AutoResp™1 v1.6.0 or AutoResp™4 v1.8.0 software (Loligo Systems, Denmark), respectively. For each experiment, four fish were individually transferred to their own respirometer in a plastic bag filled with tank water; air exposure was avoided so that gills and metabolism were not altered (Zahl et al. 2010). A fifth respirometer was used as a control. Background respirometer $\dot{M}O_2$ was measured before the fish was introduced into the respirometer and after it was removed. $\dot{M}O_2$ values were corrected for background respiration.

Standard metabolic rate (SMR)

SMR corresponds to the minimal metabolic demands required to sustain life in fasting and "resting" fish (Fry 1971; Brett and Groves 1979). There is no established method to calculate SMR. We used a quantile approach in our study because it seemed more robust than other methods proposed in the literature since it is less influenced by spontaneous activity (Daoud et al. 2007; Dupont-Prinet et al. 2010; Nelson and Chabot 2011). This approach assumes that a certain proportion of the observed $\dot{M}O_2$ values are actually below true SMR because of measurement errors and biological variability. The quantile splits the dataset into the q smallest and the 1–q largest values, where q is a proportion chosen by the experimenter. In other studies, q varied from 0.05 (Van den Thillart et al. 1994) to 0.25 (Dorcas et al. 2004). In our study, SMR was estimated for all fish by calculating the quantile (q = 0.15) of the $\dot{M}O_2$ values obtained after a 6 h acclimation period (recovery from handling stress) and for at least 42 h when fish were left undisturbed. With this setting, SMR passed through the middle of the data points in periods of low $\dot{M}O_2$.

Critical O_2 limit (O_{2crit})

This experiment was conducted on 14 adults and 12 juveniles. Oxygen uptake was measured for at least 48 h in normoxia to estimate SMR. After this initial period, DO was gradually decreased from 100 to 10% (5% sat. if required) over a period of approximately 10 h. The AutoResp™ software calculated $\dot{M}O_2$ in real time. The experiment was stopped for a given fish when it showed signs of respiratory distress, i.e., when $\dot{M}O_2$ decreased below SMR for one hour. To ensure that fish were not in extreme distress, we regularly visually checked them during this period using a red light; fish typically remained quiescent throughout. At the end of each trial, fish were anaesthetized (metomidate hydrochloride, Aquacalm^{TM}; 5 mg·L^{-1}, Western Chemical Inc., WA, USA), weighed, and FL measured. In our study, the O_{2crit} refers to the DO level below which an animal cannot indefinitely maintain its SMR, so it cannot sustain its vital functions (Wang et al. 2009). O_{2crit} was calculated using an algorithm written in R (R Development Core Team 2011) to identify data points falling below SMR and a linear regression was fit; the intersection of this line with the horizontal line represents SMR (Fig. 1).

Maximum metabolic rate (MMR) and aerobic scope (AS)

MMR corresponds to the highest measure of $\dot{M}O_2$, which is usually associated with intense exercise. AS represents the metabolic framework within which the animal must meet all its metabolic needs. We measured the impact of hypoxia on MMR and AS in juvenile fish only. In addition to normoxia (control), targeted DO levels were 35, 25, and 20% sat. However, incomplete mixing due to the large size of the rearing tank and possible drift of the galvanic Mini probe caused differences between DO measured in the ambient tank and DO in the respirometers at the end of the flush periods. DO at the end of the flush period was used to represent the DO experienced by the fish (more precisely, the experienced DO for each cycle was the average DO value from the data used to calculate $\dot{M}O_2$); the average achieved experimental levels were 28, 23, and 19% sat.

Some fishes, including Greenland halibut, do not swim in Brett-type swim tunnels. For such fish, maximum oxygen consumption can be measured following exhausting activity that requires oxygen debt repayment (Lucas and Priede 1992; Reidy et al. 1995; Liao and Lucas 2000). Juveniles were acclimated for one hour to the experimental oxygen level. They were then transferred to a circular tank (to avoid potential injuries in corners) where they were repeatedly flipped upside down until exhaustion (~15 min in normoxia and ~5 min in hypoxia), which we defined as when fish were flipped onto their backs and could no longer right themselves. The time of exhaustion was precisely recorded. To measure post-exercise $\dot{M}O_2$ and $\dot{M}O_2$ during spontaneous activity, fish were then immediately transferred into respirometer chambers. $\dot{M}O_2$ was monitored using the automatic procedure described above. After four days of recording, fish were lightly anaesthetized in metomidate hydrochloride (Aquacalm^{TM}; 5 mg·L^{-1}), weighed, and FL measured. For each fish, post-exercise $\dot{M}O_2$ was calculated. Usually, $\dot{M}O_2$ declined rapidly after the fish was placed into the respirometer. MMR was estimated using two different methods: 1) because $\dot{M}O_2$ usually declined rapidly after the fish was placed into the respirometer, the highest of the first three $\dot{M}O_2$ values was taken to represent post-exercise $\dot{M}O_2$ (Fig. 2A); 2) because juvenile Greenland halibut often displayed marked circadian cycles of $\dot{M}O_2$, the 0.99 quantile of the $\dot{M}O_2$ values recorded for each fish, excluding the first three values used to calculate post-exercise $\dot{M}O_2$, was used to estimate maximum $\dot{M}O_2$ during spontaneous activity or stress (Fig. 2B). Because activity level was not measured, the maximum value may have been caused by measurement error instead of activity. There were usually three or four values of $\dot{M}O_2$ above the 0.99 quantile, making it more likely that this estimate of high $\dot{M}O_2$ was indeed caused by spontaneous activity or stress, and not measurement error. A lower quantile was judged inadequate considering that spontaneous activity took place only occasionally in the respirometers.

The highest of either the post-exercise $\dot{M}O_2$ (20 fish) or maximum spontaneous $\dot{M}O_2$ (26 fish) was retained as our best estimate of MMR for a given fish. The rationale for using these two methods is that post-exercise $\dot{M}O_2$ measurement does not take into account circadian variations in spontaneous activity even though it is the most classical approach used in literature. Because some fish reached higher $\dot{M}O_2$ during spontaneous activity than after exercise, using both methods reduced the risk of underestimating MMR. SMR was also estimated for each fish as described previously. Aerobic scope (AS) was calculated as the difference between MMR and SMR (Fry 1971).

Specific dynamic action (SDA)

SDA corresponds to the costs of digestive processes, i.e., digestion, assimilation, and transformation of nutrients—in particular the synthesis, turnover, and accretion of proteins—in all body tissues (Jobling 1981, 1983; McCue 2006; Secor 2009; Dupont-Prinet et al. 2009, 2010). It represents one of the major metabolic costs of a fish. SDA was measured on juveniles only. We planned to study SDA at 100, 35, 25, and 20% sat. but examined the two extremes first. For the reasons given above, the average DO level ended up being 21 instead of 20%. Because of the very limited responses observed at 21% sat. relative to normoxia, the two intermediate levels (35 and 25% sat.) were dropped. To reduce stress at the beginning of each experiment, FL was measured one week before the experiment to estimate stomach volume for ration determination. Maximal food ration (g) was estimated by quantile regression (R package quantreg; Koenker

2011) of the cubic root of stomach mass as a function of fish length (cm), with q set to 0.85 (N = 9249 stomach contents collected between 1993 and 2008 in the Estuary and Gulf of St. Lawrence; D. Chabot, DFO Canada: denis.chabot@dfo-mpo.gc.ca, unpublished data, 2010): maximum stomach content mass = $(0.21455 + 0.0057 * \text{length})^3$. In this study, the food ration (capelin fillets) was set to 90% of maximum stomach content mass to avoid regurgitation. This corresponded to ~ 4% of body mass.

Greenland halibut did not eat spontaneously in the respirometer, even when they had fasted for one week. For this reason, food was offered to the fasted fish in a large tank in normoxia where they ate voluntarily. To avoid regurgitation, fish were kept in the tank for 15 min before being transferred to individual respirometers (21 or 100% sat.). $\dot{M}O_2$ was measured until it reached a stable level for at least 48 h (about 1 week). Fish were then anaesthetized with metomidate hydrochloride (Aquacalm^{TM}; 5 mg·L^{-1}) until breathing movements ceased. They were then weighed and FL measured. Unlike the other experiments, SMR was estimated from the period of stable $\dot{M}O_2$ following SDA (minimum 48 h). SDA was calculated using an algorithm written in R (R Development Core Team 2011) and the quantreg package (Koenker 2005, 2011). A non-parametric quantile regression (function rqss of quantreg) was fitted to the postprandial $\dot{M}O_2$ measurements, and SDA was deemed terminated when the fit reached SMR + 10% (Fig. 3) (Chabot and Claireaux 2008; Dupont-Prinet et al. 2010). τ was set to the value of q used to compute SMR (0.15), and therefore the same proportion of $\dot{M}O_2$ values was found below the fitted curve during SDA as during SMR. Juvenile Greenland halibut tend to be more active at night, and such bouts of activity could influence the computation of SDA parameters (see Fig. 2 and 3 for examples of nocturnal increases in $\dot{M}O_2$ likely caused by spontaneous activity). The parameter λ controls the flexibility of the fit and was set to 36 (h) to prevent this. Maximum post-prandial oxygen consumption ($\dot{M}O_{2peak}$), amplitude (difference between $\dot{M}O_{2peak}$ and SMR), time to peak (T_{peak}: time after feeding required to reach $\dot{M}O_{2peak}$), duration (time required to return to SMR + 10%), and magnitude (area under the curve) of SDA were estimated for each fish (Fig. 3).

Statistical analysis

Normality and homogeneity of variances were verified by Shapiro-Wilks and Brown-Forsythe tests, respectively. MMR and AS data were log transformed to avoid heteroscedasticity. One-way ANOVAs were used to test for the effect of developmental stage (adult and juvenile) or DO level on measures of metabolism. When appropriate, *a posteriori* Tukey tests were used to compare means ($\alpha = 0.05$). The relationship between SMR of each individual (SMR_{ind}, mg $O_2 \cdot h^{-1}$) and body mass was analyzed by linear regression (both variables were log transformed to linearize the relationship and reduce heteroscedasticity). Statistical analyses were performed with the Statistica software package (Statsoft v.6.1, Tulsa, OK, USA).

Results

SMR

SMR was significantly greater in juveniles than in adults (Table 1; $F_{[1, 24]} = 117.232$, $P < 0.001$). The relationship between SMR_{ind} and wet body mass is described by the equation $\log(SMR_{ind}) = 0.7708 \cdot \log(mass) - 1.0247$ (Fig. 4). Although this study was not designed to establish the relationship between SMR and body mass, and the entire range of body masses was not covered, the slopes obtained from each size group (0.91 ± 0.18 for juveniles and 0.98 ± 0.18

for adults, slope ± SE) did not differ significantly since their 95% confidence intervals (based on SE x 2) overlapped. The slope for both groups combined was lower but is based on a broader range of mass values, thus it is a more reliable estimate of the allometric exponent (0.77 ± 0.02). Its confidence interval also overlapped with that of the two groups taken separately.

O_{2crit}

Greenland halibut were tolerant to DO low levels, with O_{2crit} below 15% sat. (Table 1). O_{2crit} was significantly higher for juveniles than for adults (Table 1; $F_{[1, 24]} = 10.718$, $P = 0.003$), indicating that juveniles were less tolerant to hypoxia than adults.

Aerobic metabolism

The SMR of juveniles was not affected by DO levels (Table 2; $F_{[3, 42]} = 2.711$, $P = 0.057$). While MMR significantly decreased (55%) from normoxia to hypoxia (Table 2; $F_{[3, 42]} = 30.645$, $P < 0.001$), it remained similar between the different levels of hypoxia. AS in normoxia was significantly greater than at any hypoxic level (Table 2; $F_{[3, 42]} = 31.779$, $P < 0.001$). AS was similar at the two intermediate levels (23 and 28% sat.), but it was significantly smaller at 19% sat. than at any other DO level and represented only 28% of the AS value calculated in normoxia.

Specific dynamic action (SDA)

In normoxia, $\dot{M}O_2$ increased during SDA until reaching its maximum (T_{peak}) about 28 h after food intake (Table 3). In normoxia, SDA mobilized up to 24% of AS when calculated under similar conditions (20.91 *vs* 87.06 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$). The only significant effect of severe hypoxia on SDA was an increase in duration (Table 3; $F_{[1, 15]} = 4.588$, $P = 0.049$). Average SDA traces show that postprandial $\dot{M}O_2$ tended to be lower in hypoxia than normoxia for the first 72 h after food ingestion; it remained high for another 30 h but dropped rapidly after 72 h for the normoxic group. However, these differences were not significant in our experiment, as shown by the overlapping 95% confidence bands (Fig. 5). Because of the decrease in MMR, SDA mobilized between 49 and 75% of AS when calculated at similar hypoxic levels (23 and 19% sat): 18.33 *vs* 37.66 or 24.53 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$ (Tables 2 and 3).

Discussion

Critical O_2 limit

Greenland halibut exhibited an O_{2crit} below 15% sat. This compares well with hypoxia-tolerant species such as the sole, *Solea solea* (O_{2crit} ~ 12% sat.; Van den Thillart et al. 1994), and the pikeperch, *Sander lucioperca* (O_{2crit} = 11% and 28% sat., respectively, at 13°C and 28°C; Frisk et al. 2012). In these studies, the sole came from the North Adriatic Sea, which has long periods of hypoxia, and the pikeperch prefers turbid waters and lives in hypoxia during summer months and under the ice during winter. This supports our hypothesis, based on its distribution in the EGSL, that Greenland halibut is an extremely hypoxia-tolerant species. On the contrary, Atlantic cod, *Gadus morhua*, which is also found in the EGSL, is sufficiently sensitive to hypoxia to now be excluded from the deep waters of the Estuary (D'Amours 1993; Chabot and Claireaux 2008), where DO levels are below the threshold where hypoxia-induced mortality becomes significant in the laboratory (28% sat.; Plante et al. 1998).

Juvenile Greenland halibut proved to be less tolerant to hypoxia than adults. As a general rule, metabolic rate increases with body mass in fish, and, conversely, mass-specific metabolic rate decreases with mass. This may explain why we found adults to be more hypoxia-tolerant than juveniles. However, in some species such as the sharpsnout sea bream, *Diplodus puntazzo*, and largemouth bass, *Micropterus salmoides* (Burleson et al. 2001; Cerezo and García 2004), large individuals have been shown to be less hypoxia-tolerant than small individuals, despite their lower mass-specific metabolic rate. More work is therefore required to describe the mechanism underlying the differences in the hypoxia tolerance between juvenile and adult Greenland halibut.

The lower hypoxia tolerance of juvenile Greenland halibut has important ecological implications considering that the Estuary, which is more severely hypoxic than the Gulf, is considered as a nursery habitat for this species (Bourdages et al. 2010; Ait Youcef et al. in press). Field values of DO can be as little as 3% sat. above the O_{2crit} of juvenile fish: although most measures of bottom-level DO are in the 20–25% sat. range, values as low as 18% sat. have been observed (Gilbert et al. 2005, 2007) and juvenile halibut O_{2crit} was ~15% sat. Considering natural variability in both DO levels in the Estuary and in individual O_{2crit}, the most sensitive fish may already face some habitat exclusion. Our results suggest that even a small decrease in the oxygen saturation could have a major impact on the distribution of this species in the Estuary and Gulf of St. Lawrence. There is a study in progress to determine juvenile growth and stomach content of fish captured in different zones and depths of the St. Lawrence estuary that are characterized by different temperature, salinity, and DO conditions (Ait Youcef pers. comm.). The combination of physiological and ecological data will help provide a better estimation of the species' status in this environment.

Aerobic scope of juvenile Greenland halibut in normoxia

In normoxia, the AS (87 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) of Greenland halibut was very low compared to pelagic fish, such as European seabass, *Dicentrarchus labrax* (~200–300 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$; Chatelier et al. 2005, 2006), Atlantic cod, *Gadus morhua* (175 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$; Jordan and Steffensen 2007), and golden grey mullet, *Liza aurata* (~1000 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$; Milinkovitch et al. 2012); this is commonly observed in flatfishes (common sole, *Solea solea* [85 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$, Van den Thillart et al. 1994, Davoodi and Claireaux 2007; ~110 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$, Lefrançois and Claireaux 2003]; turbot, *Scophthalmus maximus* [~60 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$, Mallekh and Lagardère 2002]). This probably reflects the benthic lifestyle of flatfishes, which may rest on the bottom for several hours (although Greenland halibut feeds on pelagic prey). Although the underestimation of MMR and thus of AS remains a possibility, our estimates of AS for juvenile Greenland halibut are comparable to those of other flatfishes.

In normoxia, our study shows that SDA almost doubles $\dot{M}O_2$ relative to SMR and can mobilize up to 24% of the AS at 5°C and with a meal size close to 4% of body mass. This value is very close to results found with common sole, *Solea solea*, where the postprandial $\dot{M}O_{2peak}$ never exceeded 25% of the AS at 15°C, regardless of the size of the meal ingested (1.5 or 2.5% of body mass) and also in single-meal experiments (Couturier 2007). In contrast, the portion of AS allocated to SDA in turbot, *Scophthalmus maximus*, was temperature dependent: at 7°C, the SDA mobilized 29% of the AS compared to 52% at 16°C (Mallekh and Lagardère 2002). The AS of sole and turbot can be ~1.2 and 1.7 times higher than that of the Greenland halibut, respectively (Mallekh and Lagardère 2002; Lefrançois and Claireaux 2003). In Atlantic cod, *Gadus morhua*, or European seabass, *Dicentrarchus labrax*, which have AS values larger than flatfish, the SDA can mobilize respectively 68% and 20% of AS (Jordan and Steffensen 2007; Dupont-Prinet et al.

2010). Such variability among studies may reflect methodological differences (e.g. meal size, temperature), or species differences (e.g. inter-specific metabolic differences, pathway for nutrient handling. Such differences could also be related to the level of metabolic scope or to the proportion of the scope that can be allocated to SDA. Some species spend more time swimming than others and therefore need greater flexibility to respond to swim-muscle demands.

In Greenland halibut, aerobic capacity that is not used for digestive processes can be required for foraging activity. This species has been described as a “voracious, bathypelagic predator” (Scott and Scott 1988), and many of its important prey species are pelagic, such as capelin, redfishes, shrimp, and cephalopods (Bowering and Lilly 1992; Rodriguez-Marin et al. 1995; Jørgensen 1997; Michalsen and Nedreaas 1998; Savenkoff et al. 2007; Solmundsson 2007; Dwyer et al. 2010). Holmes and Gibson (1983) also showed that other flatfishes hunt in the water column and indicate that this was the case for 70% of attacks of the turbot *Scophtalmus maximus*. Greenland halibut require some aerobic capacity above that used for SDA to be able to feed before their previous meal is completely digested. Furthermore, it is likely that field values of $\dot{M}O_{2peak}$ regularly exceed what was observed in this study. Meals can be larger in the field, since the ration we used was about 90% of a full stomach in wild Greenland halibut. Furthermore, continuous feeding over several days increases $\dot{M}O_{2peak}$ considerably compared to a single meal (Soofiani and Hawkins 1982).

Aerobic scope of juveniles in hypoxia

The general trend of a decrease in AS as a function of ambient DO caused by a limitation of MMR agrees with previous studies in other teleosts (Jobling 1982; Schurmann and Steffensen

1992; Van den Thillart et al. 1994; Crocker and Ceck 1997; Claireaux and Lagardère 1999; Mallekh and Lagardère 2002; Jourdan-Pineau et al. 2009; Dupont-Prinet et al. 2009; Pörtner and Peck 2010). It is well known that metabolic rate is influenced by environmental factors, particularly temperature and DO level. Fry (1971) considered temperature to be a factor controlling metabolic rate and DO to be a limiting factor. Although we observed a significant decrease in MMR between normoxia and hypoxia (28, 23, and 19% sat.), we did not observe significant differences in MMR between the three levels of hypoxia even though 19% sat. is close to the critical oxygen threshold in juveniles. Biological variability and sample size could partly explain the lack of significant differences in MMR according to ANOVA. Biological variability was less pronounced with AS, where each fish served as its own control (both MMR and SMR were measured on the same fish). In this case, there was a further decline in AS at 19% sat. compared with 28 and 23%.

At 19% sat., the AS of juvenile Greenland halibut was only 28% of the AS recorded at 100% sat. Therefore, we expected both a reduction in the amplitude and an increase in the duration of SDA when juveniles were exposed to hypoxia after feeding (Jordan and Steffensen 2007; Zhang et al. 2010*b*). Though there was a tendency for $\dot{M}O_{2\mathrm{peak}}$ and thus amplitude to be lower in severe hypoxia than in normoxia, only duration was significantly altered in hypoxia: SDA lasted 25% longer at 21% sat. than at 100% sat. This finding suggests that juvenile Greenland halibut cannot process food as quickly or eat as frequently at 21% sat. as they can at 100% sat. Consequently, juvenile growth rate may be reduced at this level of hypoxia.

Although our results show little effect of severe hypoxia (21% sat.) on SDA, they indicate that Greenland halibut living in the St. Lawrence Estuary is at the edge of its metabolic capacity since the encountered oxygen level is close to the O_{2crit} for this species. The deterioration of oxygen conditions could have several consequences. Wild fish living at non-limiting DO levels are likely to feed frequently. As reported above, continuous feeding increases $\dot{M}O_{2peak}$ over what is observed with a single meal (Soofiani and Hawkins 1982). Considering that $\dot{M}O_{2peak}$ mobilized up to 75% of AS in our single-meal experiment, it is possible that $\dot{M}O_{2peak}$ would become limited by DO in fish continuously feeding in severely hypoxic conditions (Wang et al. 2009). Although this has yet to be studied, a lower $\dot{M}O_{2peak}$ should result in a longer SDA and lower overall rate of food consumption compared to normoxia.

Wild fish may avoid investing such a large proportion of their AS in SDA. In this study, fish fed freely in normoxia before being placed in hypoxia for SDA. They did not have the opportunity to adjust their ration to digest in hypoxic conditions. But in the St. Lawrence Estuary, Greenland halibut likely have to feed in severe hypoxia, even though they may capture some of their prey in the water column. Therefore, fish may adjust their food consumption to their capacity to provide the energy necessary for digestion while leaving sufficient flexibility for other needs like swimming. It has recently been demonstrated that the sea bass, *Dicentrarchus labrax,* cannot meet swimming and SDA demands simultaneously in hypoxic conditions and that it will prioritize aerobic exercise performance over SDA (Dupont-Prinet et al. 2009; Jourdan-Pineau et al. 2009). On the contrary, when temperature increases, SDA is prioritized over exercise performance in juvenile southern catfish, *Silurus meridionalis* (Pang et al. 2010). Clearly, both swimming and feeding are critical functions for survival: swimming to catch prey and feeding to provide energy and to grow. Because the cost of food assimilation increases with food intake, reducing the postprandial $\dot{M}O_{2peak}$ by reducing food ingestion is an obvious way to cope with hypoxia (Soofiani and Hawkins 1982; Mallekh and Lagardère 2002). Hypoxia usually results in decreased conversion efficiency (Stewart et al. 1967; Fry 1971; Edwards et al. 1972; Andrews et al. 1973; Brett and Blackburn 1981; Pedersen 1987; Wilhelm Filho et al. 2005; Jordan and Steffensen 2007). This will compound the impact of a reduced rate of food consumption on growth.

Environmental hypoxia has a direct influence on fisheries in two very important ways. First, hypoxia can limit the productivity of a species. Reduced growth rate and survival (because of hypoxia and increased predation) in fish could be due to the quantitative and qualitative decrease in performance because of a reduction in AS (Breitburg 2002). Greenland halibut supports an economically important fishery in the EGSL. Even though our results indicate that Greenland halibut can tolerate fairly severe hypoxia, they show that aerobic scope is greatly reduced at hypoxia levels presently encountered in the St. Lawrence Estuary and indicate that present levels of DO severely constrain the aerobic metabolism in this species. This is especially true in juveniles for which a reduction in growth rate cannot be excluded. A reduction in growth rate would be a major concern because 16% of the Greenland halibut biomass, including a high concentration of young fish aged 1 or 2 years, is found in the St. Lawrence Estuary (MPO 2010). Consequently, recruitment of this species could be affected if dissolved oxygen conditions worsened. Second, hypoxia may control species' interactions in natural populations. Indeed, hypoxia can concentrate fish prey by reducing their distribution area, which improves predation efficiency and contributes to the increase in predator numbers (Breitburg 2002; Eby et al. 2005;

Costantini et al. 2008; Zhang et al., 2010*b*; Brandt et al. 2011). This short-term positive effect of hypoxia for predators is counter-balanced by a long-term negative effect because the over-consumption of prey and the decrease of suitable habitat for predators induces a density-dependent reduction of predator growth rates and health (Breitburg 2002; Eby et al. 2005; Costantini et al. 2008; Zhang et al. 2010*a*; Brandt et al. 2011). It also results in a decrease in overall biomass and biodiversity.

Since the mid-1980s, DO levels in the deep waters of the EGSL have been stable (Gilbert et al., 2007). However, the factors responsible for the previous DO decline in the deep waters of the St. Lawrence Estuary could occur again, and possibly be enhanced by global climate changes. Thus any change in oceanographic patterns that would enhance the proportion of North Atlantic Central Water entering the Laurentian Channel, or any human-induced increase in nutrient loading in the St. Lawrence River, would result in a further deterioration in DO levels in the Estuary. This could very well impact the growth rate and/or distribution of Greenland halibut in both the Estuary and Gulf of St. Lawrence. Our results indicate the need for an ecophysiological approach in the study of the constraints imposed on fishes to improve fisheries management.

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Figure legends

Figure 1. Oxygen consumption ($\dot{M}O_2$, mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) as a function of DO (% sat.) for an individual Greenland halibut. O_{2crit} is the intercept of the standard metabolic rate (SMR, grey line) and the regression line (black line) through the points below SMR. The scatter of points at 100% sat. corresponds to data recorded during the 48 h prior to the gradual decrease in DO level, and thus include values recorded during spontaneous activity of fish.

Figure 2. Oxygen consumption ($\dot{M}O_2$, mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) over time (h:m) recorded immediately after intense exercise in Greenland halibut juveniles. Standard metabolic rate (SMR) is symbolized by the light grey line on each panel. Two estimates of maximum $\dot{M}O_2$ were calculated for each fish. First, the post-exercise maximum $\dot{M}O_2$ was estimated using data recorded within one hour (three readings) of placing the fish in the respirometer: the highest of the first three post-exercise $\dot{M}O_2$ (unfilled dot) was retained (panel A). Second, the maximum spontaneous $\dot{M}O_2$ during ≥ two days inside the respirometer was estimated as the 0.99th quantile $\dot{M}O_2$ (dark grey line, panel B). The higher of the two estimates was retained as maximum $\dot{M}O_2$ (MMR) for the fish. Grey areas in panel B indicate night periods. Note that these two examples are from two different fish.

Figure 3. Oxygen consumption ($\dot{M}O_2$, mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) over the post-feeding time in Greenland halibut juveniles (see text for details). SMR is the standard metabolic rate (in mg $O_2 \cdot h^{-1} \cdot kg^{-1}$; grey line) calculated at the end of experiment. A non-parametric quantile regression ($\tau = 0.15$, $\lambda = 36$) was fitted to the postprandial $\dot{M}O_2$ measurements until SMR + 10%. Peak (net) is the amplitude of SDA, thus $\dot{M}O_{2peak}$ (maximum post-prandial peak of $\dot{M}O_2$) is SMR + peak (net); T_{peak} (h) is the time to reach $\dot{M}O_{2peak}$; duration (h) is the time required to return to SMR + 10% after feeding;

magnitude is the total oxygen consumption during digestion (mg $O_2 \cdot kg^{-1}$). Grey areas indicate night periods.

Figure 4. Linear relationship between the standard metabolic rate of each individual (SMR_{ind}; mg $O_2 \cdot h^{-1}$) and the wet body mass (g) of Greenland halibut at 5°C. All data were log transformed. The black solid lines represent the linear regression for juveniles (on the left: $\log(SMR_{ind}) = 0.9072 \cdot \log(mass) - 1.2846$; $R^2=0.44$) and adults (on the right: $\log(SMR_{ind}) = 0.9766 \cdot \log(mass) - 1.6771$; $R^2=0.71$). The dashed line represents the overall linear regression: $\log(SMR_{ind}) = 0.7708 \cdot \log(mass) - 1.0247$ ($R^2=0.96$).

Figure 5. Mean (± 0.95% confidence interval) rates of post-prandial oxygen uptake (mg $O_2 \cdot h^{-1} \cdot kg^{-1}$) over time in normoxia (dark grey) and hypoxia at 21% sat. (light grey). The non-parametric fit for each was used to predict a value of $\dot{M}_{O_2}$ at 0.5 h intervals after feeding. The average value and its 95% confidence interval were computed for each treatment.

Table 1: Fork length (FL), mass, standard metabolic rate (SMR), and critical oxygen level (O_{2crit}) in adult (N=14) and juvenile (N=12) Greenland halibut (mean ± s.e.m.). P is the probability of a difference between adults and juveniles.

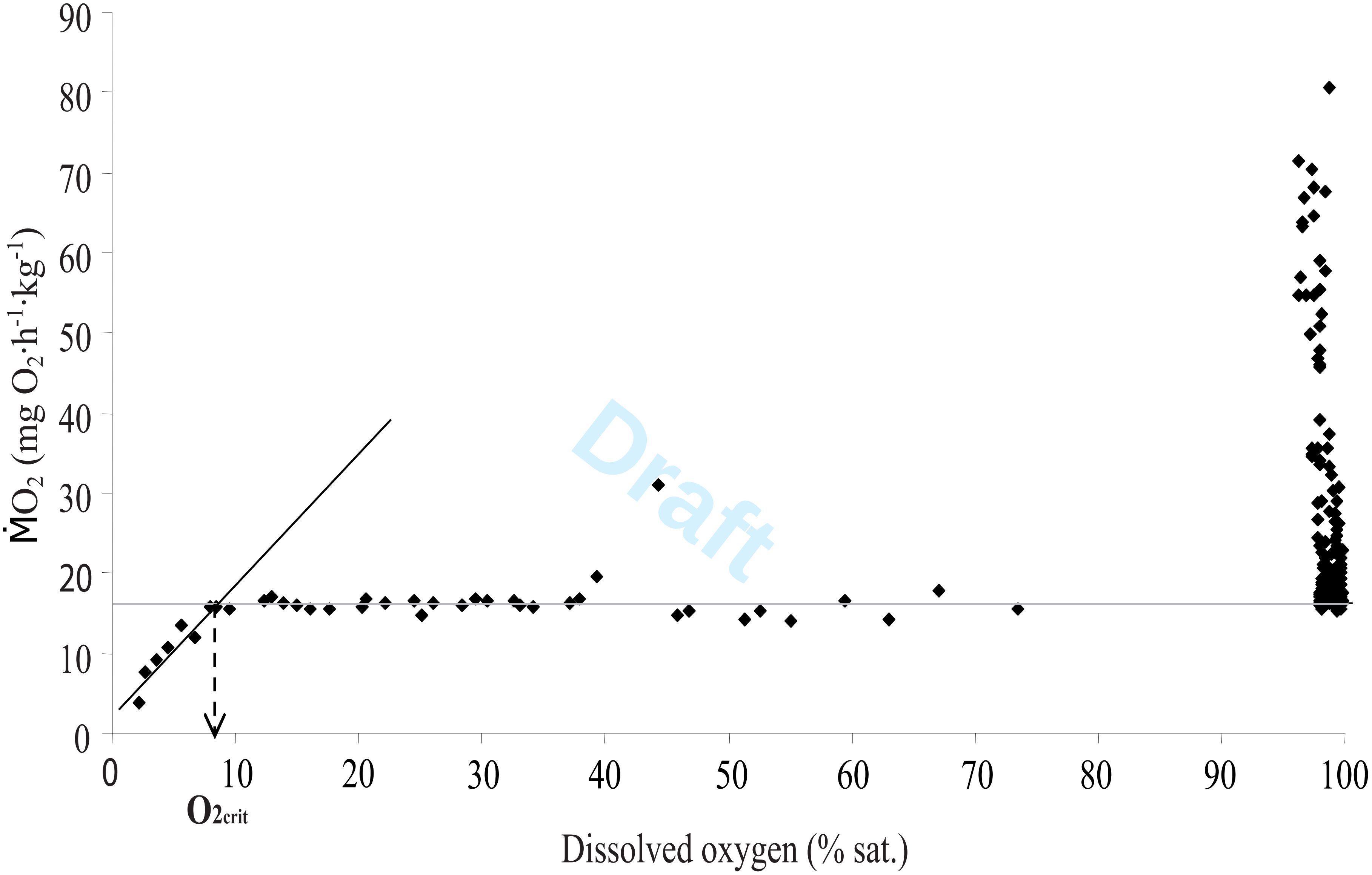
	Adults	Juveniles	P
FL (cm)	51 ± 1.1	23 ± 0.5	< 0.001
Mass (g)	1465 ± 114	91 ± 6	< 0.001
SMR (mg $O_2 \cdot h^{-1} \cdot kg^{-1}$)	18.02 ± 0.84	36.20 ± 1.53	< 0.001
O_{2crit} (% sat.)	11.10 ± 0.72	14.89 ± 0.92	0.003

Table 2: Effects of dissolved oxygen (% sat.) on aerobic metabolism in juvenile Greenland halibut. Oxygen treatments: normoxia (100% sat.) or hypoxia (28, 23, and 19% sat.), fork length (FL), standard (SMR) and maximum metabolic rate (MMR), aerobic scope (AS), (means ± s.e.m.). N=12, 11, 10, and 13 at 100, 28, 23, and 19% sat., respectively. FL, mass, and SMR were not significantly different among treatments. Within a row, means with different letters were statistically different.

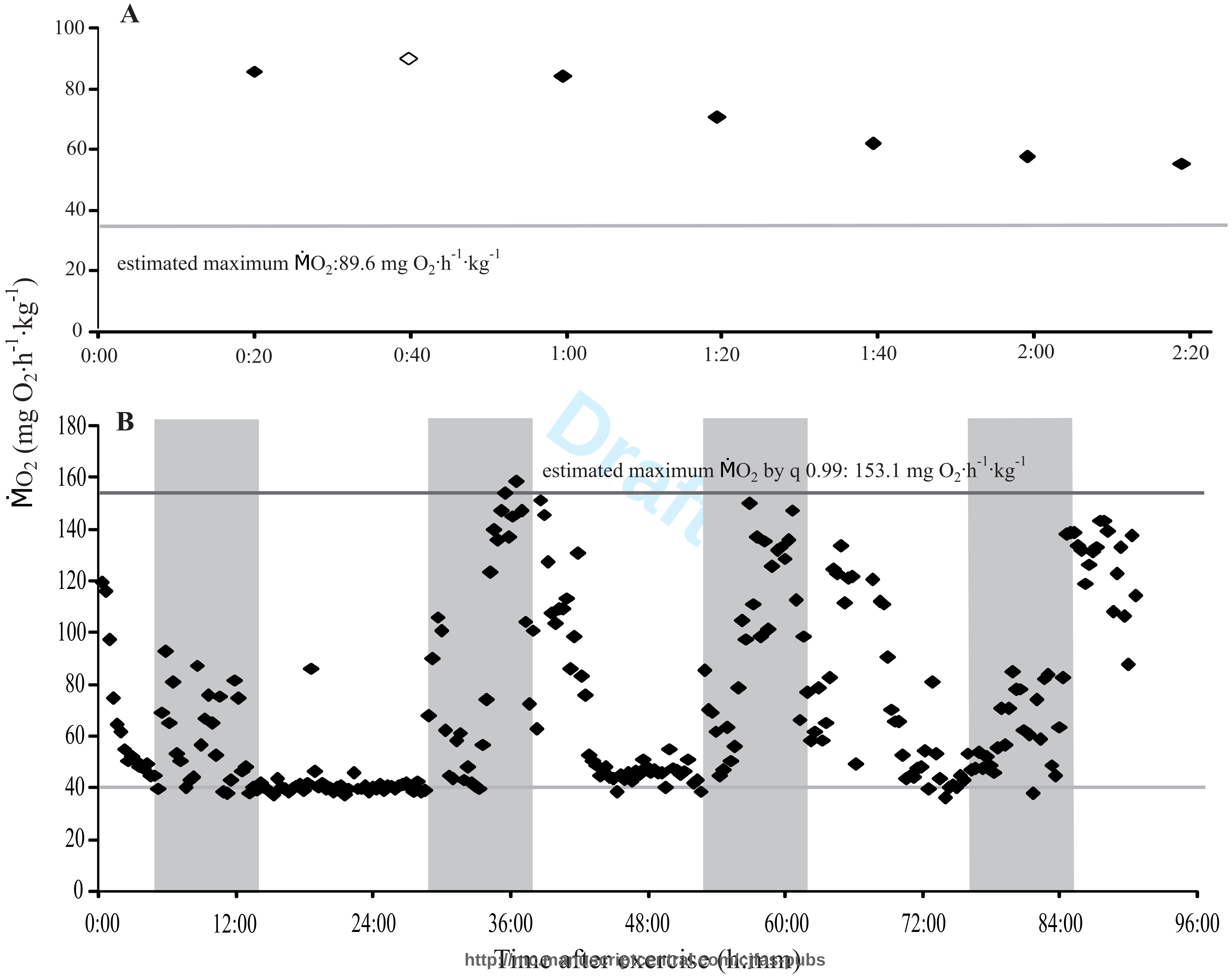
	Dissolved oxygen			
	100%	28%	23%	19%
FL (cm)	21 ± 0.2	21 ± 0.6	21 ± 0.7	21 ± 0.6
Mass (g)	80 ± 3	80 ± 8	73 ± 8	82 ± 7
SMR ($O_2 \cdot h^{-1} \cdot kg^{-1}$)	38.79 ± 1.33	29.60 ± 2.17	31.83 ± 4.12	32.00 ± 1.94
MMR ($O_2 \cdot h^{-1} \cdot kg^{-1}$)	125.85 ± 7.85 ^{b}	68.41 ± 5.23 ^{a}	66.87 ± 5.50 ^{a}	56.38 ± 2.81 ^{a}
AS ($O_2 \cdot h^{-1} \cdot kg^{-1}$)	87.06 ± 7.66 ^{c}	38.81 ± 3.82 ^{b}	35.04 ± 2.93 ^{ab}	24.38 ± 2.72 ^{a}

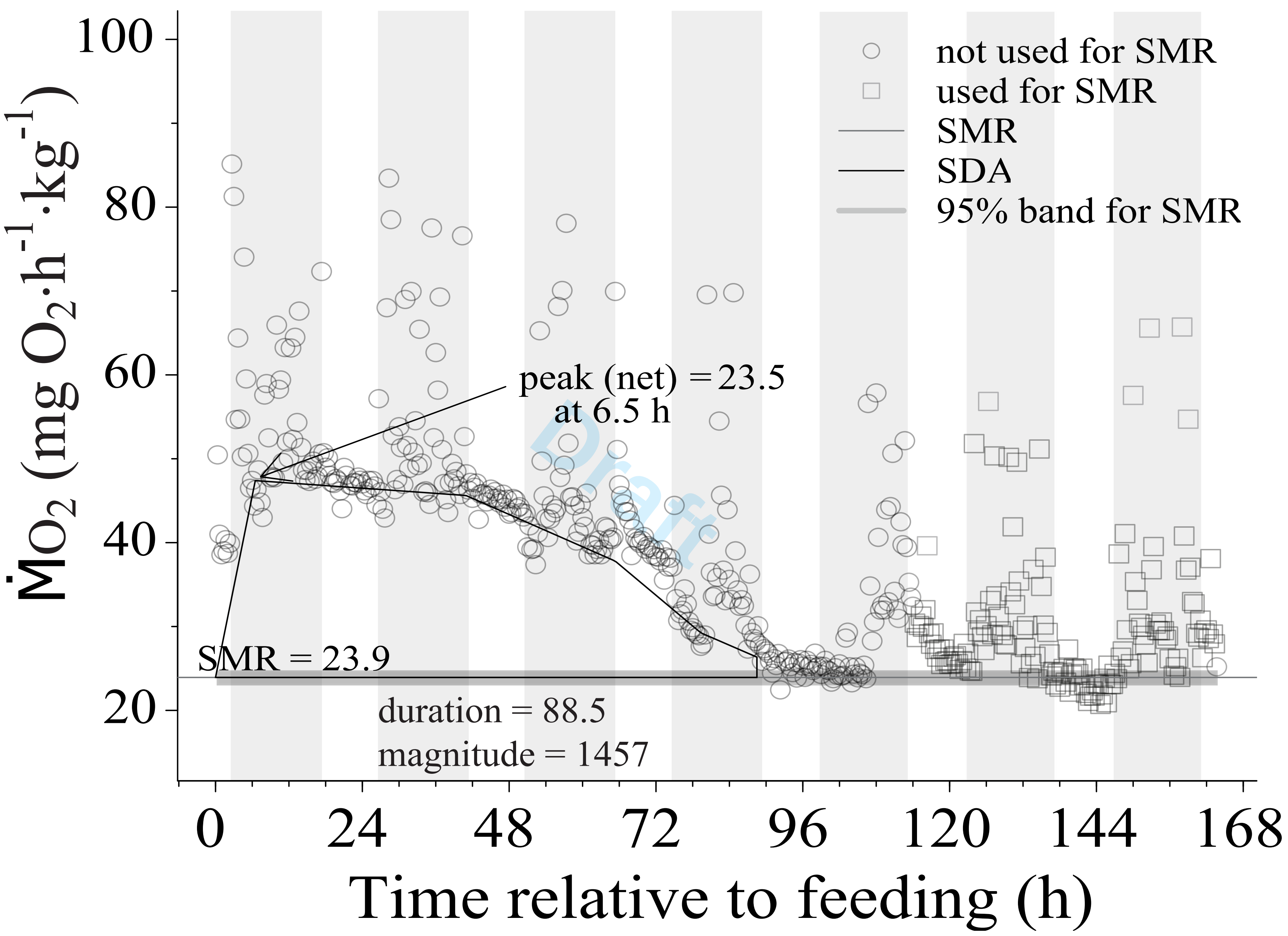
Table 3: Effects of hypoxia (21% sat.) on the postprandial metabolic response (specific dynamic action, SDA) in juvenile Greenland halibut. Mean (± s.e.m.), N=10 in normoxia, and N=7 in hypoxia. Fork length (FL); mass; SMR (mg O2·h^{-1}·kg^{-1}); $\dot{M}O_{2peak}$ is the maximum oxygen consumption observed during SDA; amplitude is the difference between peak oxygen consumption and SMR; T_{peak} is the time required to reach $\dot{M}O_{2peak}$; duration is the time required to return to SMR + 10% after feeding; magnitude is the total oxygen consumed during digestion. P is the probability of a difference between normoxia and hypoxia.

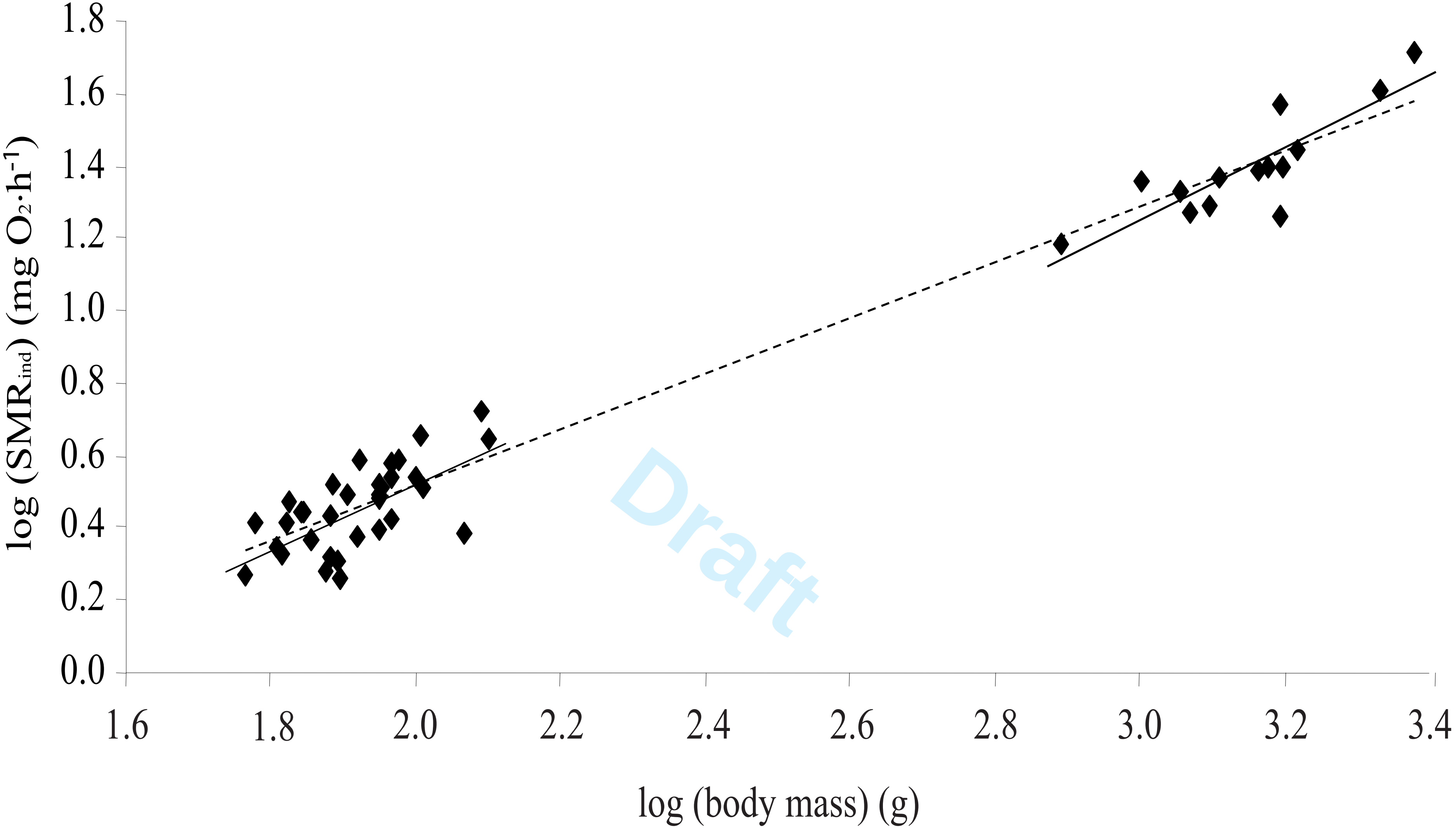
	Normoxia (100%)	Hypoxia (21%)	P
FL (cm)	21 ± 0.4	21 ± 0.3	0.247
Mass (g)	78 ± 6	72 ± 5	0.412
SMR (mg O_2·h^{-1}·kg^{-1})	33.54 ± 1.90	31.85 ± 2.39	0.585
$\dot{M}O_{2peak}$ (mg O_2·h^{-1}·kg^{-1})	54.46 ± 2.47	50.18 ± 3.43	0.314
Amplitude (mg O_2·h^{-1}·kg^{-1})	20.91 ± 1.52	18.33 ± 2.37	0.350
T_{peak} (h)	27.85 ± 6.81	47.93 ± 15.47	0.206
Duration (h)	117.70 ± 8.69	147.14 ± 10.77	0.049
Magnitude (area under the curve mg O_2·kg^{-1})	1507.24 ± 79.56	1736.12 ± 158.26	0.178



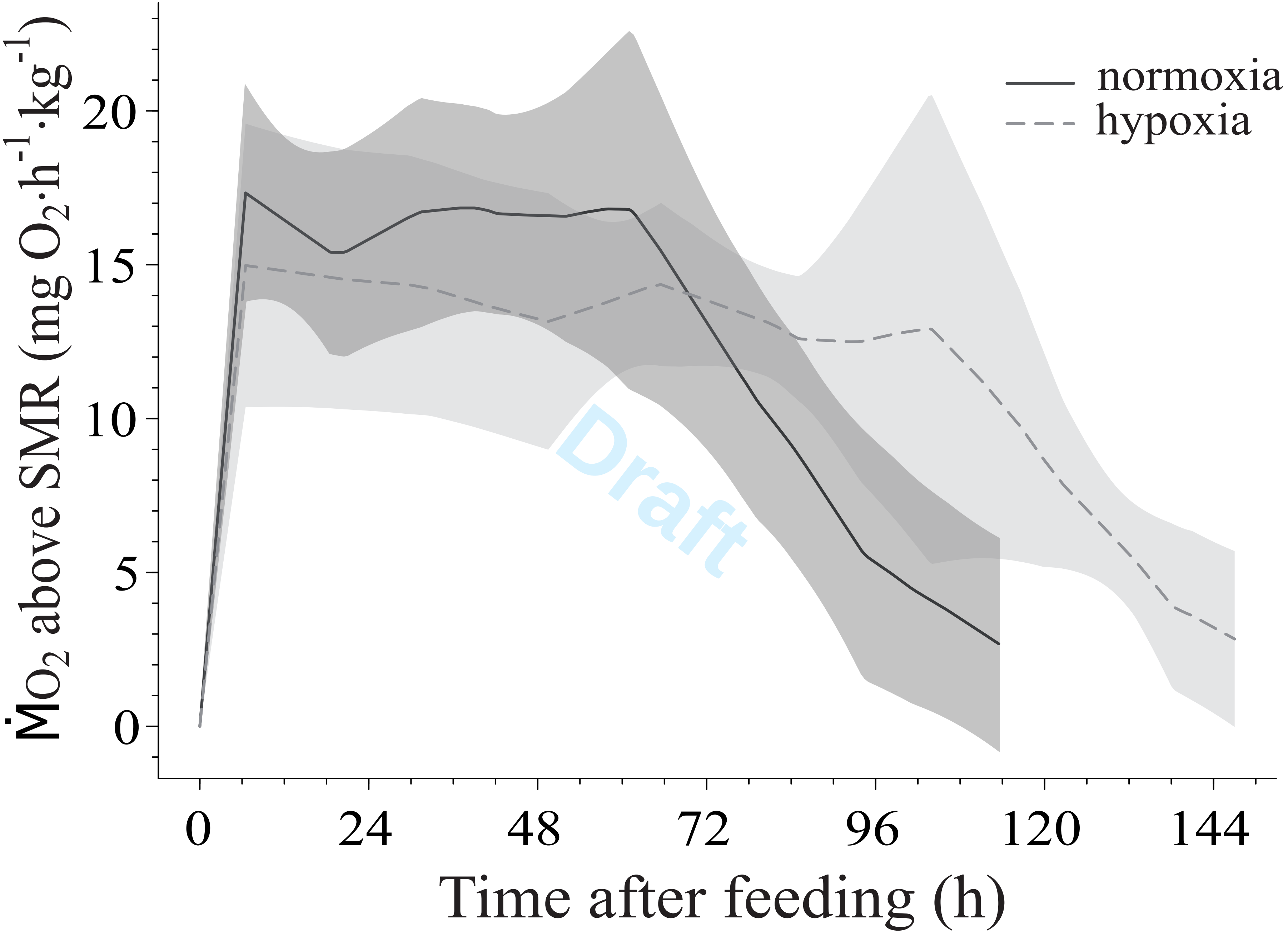
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
$\dot{M}O_2$ (mg $O_2 \cdot h^{-1} \cdot kg^{-1}$)
O_{2crit}
Dissolved oxygen (% sat.)


A
100
80
60
40
20
0
0:00
0:20
0:40
1:00
1:20
1:40
2:00
2:20
estimated maximum $\dot{M}O_2$:89.6 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$
$\dot{M}O_2$ (mg $O_2 \cdot h^{-1} \cdot kg^{-1}$)
B
180
160
140
120
100
80
60
40
20
0
0:00
12:00
24:00
36:00
48:00
60:00
72:00
84:00
96:00
estimated maximum $\dot{M}O_2$ by q 0.99: 153.1 mg $O_2 \cdot h^{-1} \cdot kg^{-1}$
Time after exercise (h:mm)


not used for SMR
used for SMR
SMR
SDA
95% band for SMR
peak (net) = 23.5
at 6.5 h
SMR = 23.9
duration = 88.5
magnitude = 1457
$\dot{M}O_2$ (mg O_2·h^{-1}·kg^{-1})
100
80
60
40
20
0
24
48
72
96
120
144
168
Time relative to feeding (h)



1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
log (SMR$_{ind}$) (mg O$_2$·h^{-1})
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
log (body mass) (g)


normoxia
hypoxia
$\dot{M}O_2$ above SMR (mg $O_2 \cdot h^{-1} \cdot kg^{-1}$)
0
5
10
15
20
0
24
48
72
96
120
144
Time after feeding (h)