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BRIEF COMMUNICATIONS

Excess post-hypoxic oxygen consumption in Atlantic cod *Gadus morhua*

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Atlantic cod *Gadus morhua* experienced oxygen deficit (D_{O_2}) when exposed to oxygen levels below their critical level (*c*. 73% of p_{crit}) and subsequent excess post-hypoxic oxygen consumption (C_{EPHO}) upon return to normoxic conditions, indicative of an oxygen debt. The mean ± S.E. C_{EPHO}:D_{O_2} was 6·9 ± 1·5, suggesting that resorting to anaerobic energy production in severe hypoxia is energetically expensive.

Key words: acute severe hypoxia; anaerobic metabolism; ATP; lactate; oxygen debt.

Tagging of Atlantic cod *Gadus morhua* L. 1758 in the Bornholm Basin (Baltic Sea) has shown that some individuals (approximately one third of the tagged population) voluntarily dive into severely hypoxic bottom water, experiencing oxygen partial pressures (P_{O_2}) as low as 2·1 kPa (*c*. 10% air saturation). These fish altogether spend 7% of their total time at $P_{O_2} \leq 4{\cdot}2$ kPa (*c*. 20% air saturation) (Neuenfeldt *et al*., 2009), which falls below their critical level (p_{crit}) reported as ranging between 4·3 and 4·8 kPa at 10° C (*c*. 20·5 and 23% air saturation) (Schurmann & Steffensen, 1997; Herbert & Steffensen, 2005). Overall, residence times were strongly correlated with the prevailing degree of hypoxia, for example, 90 min at 7·3 kPa (*c*. 35% air saturation) or 70 min at 4·2 kPa (20% air saturation), after which the fish returned to well-oxygenated water (Neuenfeldt *et al*., 2009). Such excursions are probably related to foraging, as supported by previous reports of benthic food items in the stomachs of *G. morhua* in the same area (Neuenfeldt & Beyer, 2003). Comparable behaviour has been observed in other species. For example, the scalloped hammerhead shark *Sphyrna lewini* (Griffith & Smith 1834) preys on deep-water squid in hypoxic water (Jorgensen *et al*., 2009); similarly, the eastern Pacific sailfish *Istiophorus platypterus* (Shaw 1792) and eastern Atlantic sailfish *Istiophorus albicans* (Latreille 1804) forage in distinct strata of cold hypoxic water in the eastern

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tropical Pacific Ocean, a strategy that may enhance their growth potential compared with western conspecifics (Prince & Goodyear, 2006). If *G. morhua* forage in hypoxic waters at oxygen levels below their p_{crit}, it is likely that they will rely on anaerobic metabolism resulting in a lactate accumulation that has to be cleared by excess oxygen consumption (oxygen debt) upon return to more well-oxygenated water (McKenzie *et al*., 2000; Speers-Roesch *et al*., 2012). Studies on turbot *Scophthalamus maximus* (L. 1758) and rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) have indicated that repayment of a post-hypoxic oxygen debt is expensive as indicated by measurements of a subsequent excess post-hypoxic oxygen consumption (C_{EPHO}; the increase in oxygen consumption in normoxia after a fish has been exposed to severe hypoxia) (Maxime *et al*., 2000; Svendsen *et al*., 2012). This study quantified the oxygen deficit (D_{O_2}; the oxygen consumption in severe hypoxia) that *G. morhua* experience during 40 min exposure to mean ± S.E. P_{O_2} of 3·3 ± 0·1 kPa (*c*. 16% air saturation), *i.e*. below their p_{crit} (*c*. 73% of p_{crit}) and, subsequently (upon return to normoxic water), the C_{EPHO}.

Gadus morhua (body mass 328–500 g) were trawled in the Sound between Denmark and Sweden (55° 45′ N; 12° 45′ E) and kept at the Marine Biological Section in 10° C, fully aerated, re-circulated sea water. Fish were acclimated for a minimum of 3 weeks and were fed dead clupeids every second day (*c*. 5% of body mass) until 5 days prior to experiments. The oxygen consumption rate (M_{O_2} mg O_2 h^{-1} kg^{-1}) was determined using intermittent flow-through respirometry (Steffensen, 1989) on individual fish ($n = 7$) placed in a 5·3 l plexiglas respirometer and using Aquaresp software (www.Aquaresp.com). A respirometric loop lasted 11·5 min with 5 min flushing, 1·5 min waiting and 5 min measuring. Background respiration was measured before and after each experiment and accounted for in the M_{O_2} calculations. For further details on formula for M_{O_2} calculations, see Behrens *et al*. (2006). To maintain a stable temperature (10° C) the respirometer was submerged in a temperature-controlled water bath. A camera was used to monitor the fish and the whole set-up was sheltered from the surroundings to assure minimum disturbance of the fish.

Each experiment consisted of three phases [see Fig. 1 and Svendsen *et al*. (2012) for details]: Phase 1 was an *c*. 48 h period during which the fish was in normoxia [$P_{O_2} \geq 19{\cdot}9$ kPa (≥95% air saturation)]. After the fish calmed down from handling, standard metabolic rate (R_S) was determined as the mean value associated with the first modal distribution of a double normal distribution (Steffensen *et al*., 1994) fitted to the M_{O_2} measurements achieved during the last 3 h of the period. During phase 2, the fish was exposed to acute hypoxia [mean ± S.E. = 3·3 ± 0·1 kPa (*c*. 16% air saturation)], *i.e*. below p_{crit} (*c*. 73% of p_{crit}) (Schurmann & Steffensen, 1997; Herbert & Steffensen, 2005) for 40 min (0·66 h). Pilot experiments showed that longer exposure to severe hypoxia resulted in struggling behaviour. Acute severe hypoxia inside the respirometer was achieved by deoxygenating the external water with a stream of nitrogen while the respirometer was in its measuring state (*i.e*. closed), whereafter the severely hypoxic water entered the respirometer during the flush (open) period (5 min). In this way, the respirometer P_{O_2} was decreased from normoxia to the desired hypoxia level within one single flush period. During the measuring period (phase 2), the P_{O_2} dropped from the initial *c*. 16 to 13–14% air saturation on average. Phase 3 was subsequent to severe hypoxic exposure, with the fish in normoxia [$P_{O_2} \geq 16{\cdot}8$ kPa (≥80% air saturation)], created by an equivalent procedure to deoxygenation, except by bubbling with oxygen. Phase 3 lasted

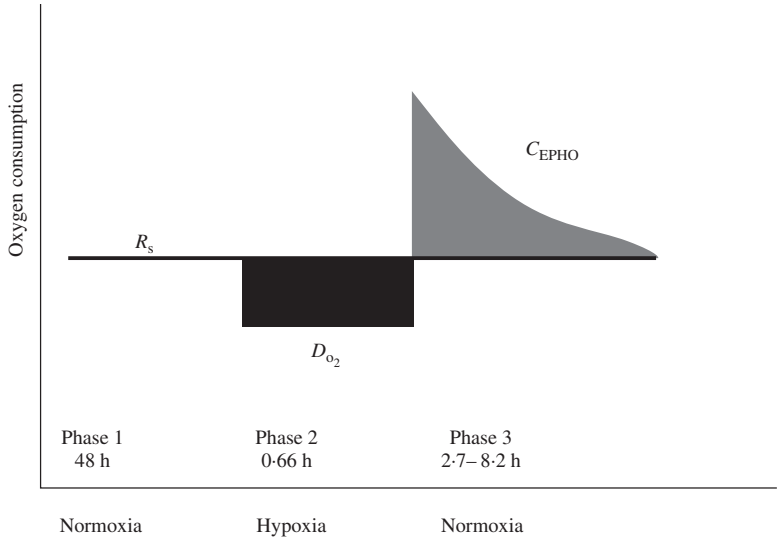

FIG. 1. Schematic figure of oxygen consumption in relation to water oxygen partial pressure. ——, the standard metabolic rate (R_S) obtained in fully oxygenated water during phase 1. In phase 2, ■, the oxygen deficit (D_{O_2}) during exposure to hypoxia; in phase 3, ■, the excess post-hypoxic oxygen consumption (C_{EPHO}) upon subsequent return of the fish to normoxia.

for a maximum of 8·2 h. Termination of the C_{EPHO} was considered to be when two consecutive M_{O_2} measurements were below $R_S + 5\%$, as in Svendsen *et al*. (2012). Background respiration was <2·3% of R_S and the coefficient of determination (R^2) associated with each M_{O_2} measurement was >0·96 for all fish.

The D_{O_2} and C_{EPHO} were calculated as: $D_{O_2} = \sum_{i=1}^{n}[R_S - M_{O_2}(\text{measurement}-i)]t^{-1}$ and $C_{EPHO} = \sum_{i=1}^{n}\left[M_{O_2}(\text{measurement} - i) - R_S\right]t^{-1}$, where n is the number of measurements, $t = 60$ (loop time)$^{-1}$, because M_{O_2} has units mg O_2 h^{-1} kg^{-1}. For D_{O_2}, n is the total number of measurements carried out in phase 2, whereas for C_{EPHO}, n is the number of measurements within the C_{EPHO} phase as defined above. During C_{EPHO}, extreme outliers (see Fig. 2 for example) were removed manually and replaced by the mean of the two adjacent values. This never exceeded one measure per individual C_{EPHO} period.

The results are summarized in Table I. The mean ± S.E. C_{EPHO}:D_{O_2} was 6·9 ± 1·5, suggesting that in *G. morhua* the use of water with oxygen levels below the estimated p_{crit} is energetically costly. The increase in oxygen consumption after exposure to severe hypoxia indicates that *G. morhua* shifts from aerobic to anaerobic metabolism. The C_{EPHO} lasted 4·8 ± 0·7 h with a maximal M_{O_2} $\left(M_{O_2\,max}\right)$ of 128 ± 11 mg O_2 kg^{-1} h^{-1} (means ± S.E.).

Only two other studies have investigated the energetic costs associated with exposure to oxygen levels below p_{crit}. In both of these studies, the C_{EPHO}:D_{O_2} was substantially higher than the 6·9 for *G. morhua* following exposure to P_{O_2} of 3·3 kPa. Maxime *et al*. (2000) reported an C_{EPHO}:D_{O_2} of 16 for *S. maximus*, and Svendsen *et al*. (2012) reported a ratio of 30 for *O. mykiss* following exposure to

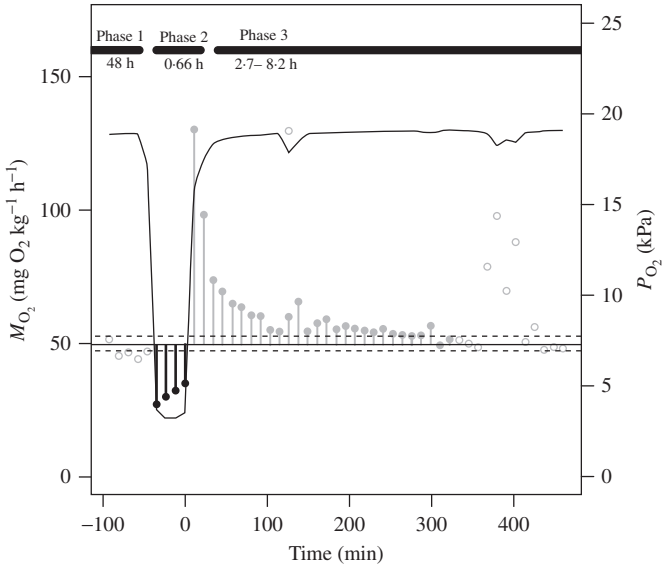

FIG. 2. An example of the oxygen consumption rate (M_{O_2} mg O_2 kg^{-1} h^{-1}) before, during and after exposure to acute severe hypoxia [3·3 kPa (*c*. 16% air saturation)] for a 443 g *Gadus morhua*. ——, standard metabolic rate (R_S, mg O_2 kg^{-1} h^{-1}) of the fish obtained during phase 1; – – , $R_S \pm 5\%$; ↓, difference between M_{O_2} and R_S during exposure to severe hypoxia (phase 2), *i.e*. the values used to calculate the oxygen deficit (D_{O_2}) (see also Fig. 1). In phase 3, ↑, M_{O_2} of the subsequent return of the fish to normoxic water. These measurements were used to calculate the excess post-hypoxic oxygen consumption (C_{EPHO}). ⁓, the oxygen partial pressure (P_{O_2}, kPa) of the water. The end of the C_{EPHO} phase was defined as the time when two consecutive M_{O_2} measurements were below the $R_S + 5\%$. ○, measurements that were not used for calculations of C_{EPHO}. One measurement (○ at *c*. 130 min subsequent return to normoxia) was considered an outlier (presumably a result of spontaneous activity) and removed manually from the calculation of C_{EPHO}.

2·7 and 2·1 kPa, respectively. The interspecific trend suggests a negative correlation between the level of P_{O_2} and C_{EPHO}:D_{O_2}. The comparison, however, is limited by the number of species and also differences in the protocols used. The % of p_{crit} that *G. morhua* and *O. mykiss* (Svendsen *et al*., 2012) were exposed to was very similar, 73 and 74% of p_{crit}, respectively. *Scophthalamus maximus*, however, was exposed to progressive hypoxia for 1 h (P_{O_2} ranging from 66% of and nearly up to p_{crit}) and increasing plasma and muscle lactate concentrations were evident from 8 kPa onwards, well before any reduction in M_{O_2} at p_{crit} (4 kPa). This indicates an anaerobic component well before p_{crit} is reached in *S. maximus* and, if this is the case, the M_{O_2} measures underestimate the actual energy use by the fish during this period. Notably, this anaerobic component is not included in the D_{O_2} but in C_{EPHO} when the lactate is cleared upon return to normoxia. This may explain, at least in part, the increased C_{EPHO}:D_{O_2} in *S. maximus* (16) compared with *G. morhua* (7). Furthermore, exposure time for *S. maximus* was longer (1 h) than for *G. morhua* (0·66 h) as was the exposure time for *O. mykiss* (0·97 h), and *S. maximus* was tested at the highest temperature (17° C), which increases R_S and sensitivity to hypoxia (Schurmann & Steffensen, 1997).

TABLE I. Summary of results following exposure of *Gadus morhua* ($n = 7$) to severe acute hypoxia [mean ± S.E. P_{O_2} of 3·3 ± 0·1 kPa (*c.* 16% air saturation)] for 40 min and subsequent return to normoxia

	Mean ± S.E.
R_S (mg O_2 kg^{-1} h^{-1})	53 ± 2
D_{O_2} (mg O_2 kg^{-1})	10·6 ± 0·8
C_{EPHO} (mg O_2 kg^{-1})	69·9 ± 15·6
C_{EPHO}:D_{O_2}	6·9 ± 1·5
$M_{O_2\,max}$ (mg O_2 kg^{-1} h^{-1})	128 ± 11
C_{EPHO} duration (h)	4·8 ± 0·7

R_S, standard metabolic rate; D_{O_2}, oxygen deficit; C_{EPHO}, excess post-hypoxic oxygen consumption; $M_{O_2\,max}$, maximum oxygen consumption after exposure to acute severe hypoxia (*i.e.* during phase 3).

Assuming that the difference between oxygen consumption in normoxia and severe hypoxia is compensated for by anaerobic metabolism (McKenzie *et al.*, 2000; Speers-Roesch *et al.*, 2012), differences in C_{EPHO}:D_{O_2} may indeed occur between species, possibly explained by differences in lactate dynamics (Milligan & Girard, 1993). During recovery from severe hypoxia (*i.e.* below p_{crit}) pathways are activated to clear excess lactate in blood and tissues (Plante *et al.*, 1998; Maxime *et al.*, 2000) and re-synthesize ATP, creatine phosphate and glycogen storages (Mandic *et al.*, 2008). During glycogenolysis, 1 mol of glycogen yields 2 mol of lactate, which each contributes 1·5 mol of ATP in the anaerobic pathway. The reverse process of converting 1 mol of lactate to glycogen (glycogenesis) upon return to normoxia uses 2·5 mol of ATP (Moyes *et al.*, 1992). Three moles of ATP is thus gained at the expense of 5, which results in a repayment rate of 1·66 (5/3) when returning to normoxia. Assuming that D_{O_2} is paid only by glycogenesis, this only accounts for 18 mg O_2 kg^{-1} h^{-1} [11 mg O_2 kg^{-1} h^{-1} (*i.e.* D_{O_2}) × 1·66] of the 70 mg O_2 kg^{-1} h^{-1} measured as C_{EPHO}. The remaining 52 mg O_2 kg^{-1} h^{-1} (74%), therefore, has to be explained by other processes. Probably, some is used for restoration of creatine phosphate and ATP stores, re-establishing of ion, acid–base and fluid volume homeostasis and increased cardioventilatory work (Scarabello *et al.*, 1991; Wood, 1991).

Part of the C_{EPHO}:D_{O_2} may also be due to the (unavoidable) constraint related to working with confined experimental animals. Involuntary exposures of (any) fish to severe hypoxia probably cause a stress reaction. The present low R_S (mean ± S.E. 53 ± 2 mg O_2 kg^{-1} h^{-1}) was slightly lower than previously reported (Schurmann & Steffensen, 1997; Claireaux *et al.*, 2000), indicating that the fish were unstressed in normoxia. Furthermore, no avoidance response was observed when *G. morhua* was exposed to 40 min of severe hypoxia. Herbert & Steffensen (2005) found that exposure of *G. morhua* to progressive hypoxia resulted in increased plasma cortisol levels when P_{O_2} fell below p_{crit} (Herbert & Steffensen, 2005), and this may also occur when the fish are exposed to acute hypoxia. Consequently, a proportion of the C_{EPHO} may be associated with the costs of clearing cortisol (and perhaps other stress hormones) from the blood. As *O. mykiss* are considered rather sensitive and easily agitated fish known to show avoidance reactions when exposed to decreasing oxygen levels (Vianen *et al.*, 2001), part of the C_{EPHO} ratio discrepancy between

G. morhua and *O. mykiss* may be explained by higher levels of stress during exposure to severe hypoxia in *O. mykiss*.

Assuming that the present C_{EPHO}:D_{O_2} applies to foraging *G. morhua*, what is the additional cost for an individual undertaking foraging excursions into severely hypoxic water? Swimming at 0·5 body lengths s^{-1} (the average swimming speed suggested to be used by foraging *G. morhua*; Løkkeborg, 1998; Løkkeborg & Fernö, 1999; Fernö *et al*., 2011) will add an extra cost of 10–15 mg O_2 kg^{-1} h^{-1} to R_S (Schurmann & Steffensen, 1997; Melzner *et al*., 2009), and it is then possible to calculate and compare the energetic cost (R_S plus the additional cost of swimming) of foraging for 24 h in normoxia to the energetic cost of foraging for 23 h in normoxia plus 1 h in severe hypoxia (below p_{crit}). The additional cost per day of spending 1 h in hypoxia is *c*. 15%; a hypoxic excursion of 1 h during the day may hence be energetically affordable if food intake (assuming equal quality in the normoxic and hypoxic habitats) is increased by >15% compared with a foraging strategy without these excursions. Furthermore, with C_{EPHO} for *G. morhua* lasting 4·8 ± 0·7 h (mean ± S.E.) it would be possible for the fish to undertake up to four dives into severe hypoxia per day with a full recovery between dives. Considering that digestion of a meal constituting 5% of the body mass of *G. morhua* occupies 55% of its aerobic metabolic scope (the difference between the maximum aerobic metabolic rate and the R_S; Jordan & Steffensen, 2007), however, it is nevertheless likely that the increased M_{O_2} following prey ingestion (the specific dynamic action, the energy expended on all activities of the body incidental to the ingestion, digestion, absorption and assimilation of a meal) might compromise, or be compromised by, any further excursions into hypoxic water while a meal is being digested.

The eastern Baltic *G. morhua* stock has recently started to recover in numbers, however, individual fish are reported much leaner, which probably reflects a decline in abundance of their main pelagic prey, sprat *Sprattus sprattus* L. 1758 and herring *Clupea harengus* L. 1758 (Eero *et al*., 2012). Such food limitation in the normal (normoxic) habitat of *G. morhua* may result in selection for alternative foraging strategies and it may be that increasing numbers of *G. morhua* resort to easily accessible, though energetically less rewarding, bottom-dwelling zoobenthos in the severely hypoxic bottom waters. This is supported by recent stomach content analysis from the Bornholm Basin (eastern Baltic Sea) (B. Huwer, unpubl. data) showing either benthic invertebrate food in the stomachs of cod or empty stomachs. Thus, as the abundance of *G. morhua* increase regionally concurrent with diminishing spatial overlap with its clupeid prey, one may expect that the fraction of cod showing this behaviour will also increase.

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